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CLINICAL AND EXPERIMENTAL

EARLY INFANTILE PROGRESSIVE MUSCULAR ATROPHY (WERDNIG-HOFFMANN) A CLINICAL AND PATHOLOGIC STUDY OF TWO CASES*

BY CHARLES E. NIXON, M.D., AND JEAN OLIVER, M.D., SAN FRANCISCO, CALIF.

THE Werdnig-Hoffmann type of progressive muscular atrophy is a comparatively rare disease; a search of the literature shows a record of about twenty typical cases, and of these only ten were studied from both the clinical and pathologic side. There is therefore still considerable uncertainty, not only as to the classification of the different types of muscular atrophy in infants, but even as to whether or not the syndrome described by Werdnig[1] and by Hoffmann[2] is a clinical entity. In a recent paper Huenekens and Bell[3] come to the conclusion from a review of the literature and a study of the case reported by them that amyotonia congenita (Oppenheim) and infantile spinal progressive muscular atrophy (Werdnig-Hoffmann) are extreme types of the same disease and that they are probably both related to the groups of myopathies represented by Erb's juvenile form of muscular dystrophy and the hereditary form of Leyden and Möbius.

Another obscure point which the recorded cases have not made clear is the relation of degeneration in the cardiac muscle to the extensive lesions that occur in the skeletal system. Globus[4] has recently reported an instance of cardiac involvement in a case of progressive muscular dystrophy and has reviewed the literature of this phase of the subject. He found a fragmentation of the muscle cells with multiplication of their nuclei and an infiltration of the interstitial tissue with fibroblasts. In the descriptions of the older writers the heart was either not examined or no detailed descriptions are given of the microscopic findings.

In the first case reported in this paper the spinal cord and muscular

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involvement are of the characteristic type without any lesions in the heart muscle. The second case shows a remarkable type of acute cell change in the anterior horn cells and definite and equally acute involvement of the heart muscle.

CASE 1.—V. O., aged three months. The family history is negative except that one well developed child was stillborn. The birth was spontaneous by breech presentation but not prolonged. A marked deformity of the chest was present from birth and respiration was diaphragmatic and abdominal. The arms were paralyzed, the right more seriously than the left. There was a double wrist-drop with contractures. Otherwise the limbs were flaccid. The right foot was in the position of a moderate equinovarus and the left in a moderate calcaneovalgus. At the time of examination in the Children's Hospital, when the child was two and one-half months old, the deep reflexes were entirely absent. There was no glandular enlargement and the von Pirquet and Wassermann tests were negative. The blood count was normal. The x-ray plate of the skull was negative.

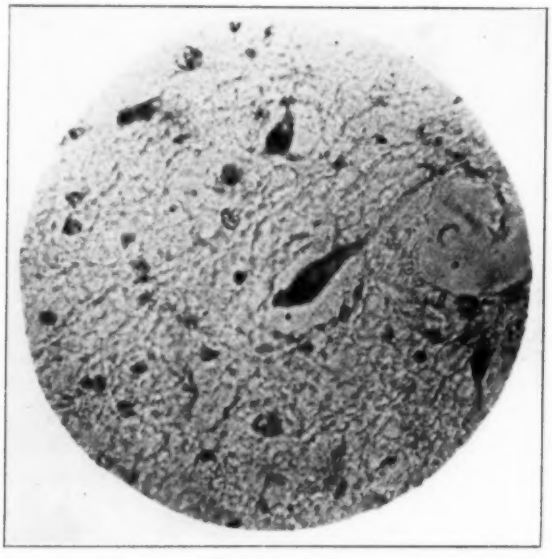

Fig. 1. (Case 1.) Anterior horn cell.

The essential necropsy findings are as follows: The body is that of a poorly developed and nourished white male infant of three months. The pectoral muscles are firm and apparently fibrous. The upper half of the chest is small and depressed. The lower half shows marked flarings of the costal margin. The costochondral junctions are fairly prominent. The muscles of the extremities are all atrophic. The arms are partially flexed and complete extension of the elbows is not possible. The thymus is fairly large. The skull was not opened.

Microscopic Examination.—The anterior horn cells of the cord are strikingly smaller than normal; there is not, however, a definite diminution in number. The cell changes are most marked in the cervical and thoracic regions but are also present in the lower cord. In the thoracic cord the lateral cells belonging to the visceromotor groups are much more normal in appearance than the other anterior horn cells.

The type of cell change is uniform throughout the cord and the extent of the cell alteration varies only to a moderate degree. The characteristic anterior horn cell is spindle shaped, the nucleus is relatively large and pale, the chromatin substance is more or less clumped, and as a rule at one end of the cell (Fig. 1). The anterior horn cells of the lum-

bar cord show less change; the cells are larger and somewhat resemble a normal cell but the nucleus is comparatively large and pale and the cytoplasm and arrangement of the Nissl bodies definitely varies from the normal. The chromatin substance is either in a few large clumps or is situated peripherally, leaving the cytoplasm largely clear.

Fig. 2. (Case 1.) Anterior roots, Weigert myelin sheath stain.

Fig. 3. (Case 1.) Posterior roots, Weigert stain.

The cells of Clarke's column show moderate chromatolytic changes. The nucleus is eccentrically placed, often appearing to be partly extruded from the cells. The chromatin material tends to be clumped in one end of the cell; in a few cells the chromatolysis is extreme so that the cell consists of a swollen nucleus partly extruding from a small amount of granular cytoplasm.

There are no special changes in the glia tissue; satellites and the so-called neuronophagia are occasionally noted, somewhat more in Clarke's column than in the anterior horn. No gitter-zellen are seen.

Weigert sections of the cord show a fairly marked degeneration of the anterior roots; the posterior roots are normal (Figs. 2 and 3.)

The somatic muscles are extremely involved (Fig. 4). There is great variation in the size of the fibers. The fasciculae may be made up wholly of small fibers or of both large and small fibers. In some of the muscle sections the cross striations are indistinct. Vacuolization is occasionally seen. Pigmentation is present in some of the bundles and varies from yellowish to yellowish brown in color. The heart muscle appears normal.

CASE 2.—We are indebted to Drs. F. Sylvester and Langley Porter for the details of the clinical history of the case.

A Japanese baby, five months old, was admitted to Lane Hospital on November 1st. It had been well until two weeks previously when it was noticed that its legs were swollen.

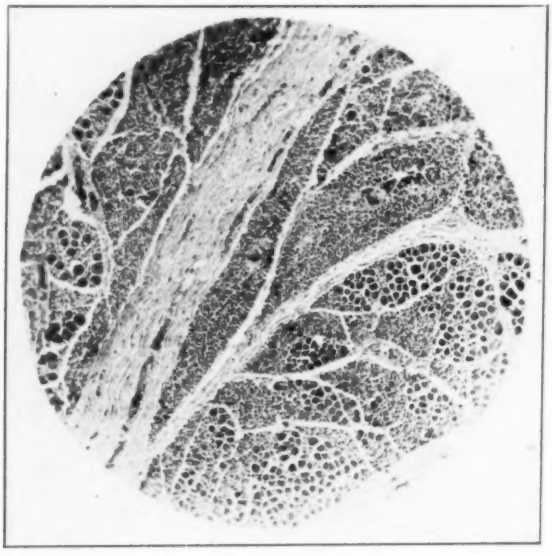

Fig. 4. (Case 1.) Skeletal muscle.

Shortly after this time Dr. Sylvester was called and noted a flaccid paralysis of practically all the muscles below the head. Even the muscles of deglutition were affected, making tube feeding necessary. A week later the child developed difficulty in breathing. On admission to the hospital the baby was limp and pale and frothing at the mouth. It rallied somewhat after a mustard bath, but the dyspnea increased and the child died the morning following its entrance to the hospital. The urine was normal and there was no disturbance in the digestive tract. A diagnosis of progressive muscular atrophy was made.

Pathologic Examination.—The necropsy was performed three hours after death. The body was that of a rather emaciated normally formed male child of normal size for its age. There was a marked atrophy of the subcutaneous fat. There was a moderate elastic edema of the palms of the hands and the soles of the feet and a marked edema of the scrotum. No special atrophy of any group of muscles was evident, but sections of the muscles in various places showed a definite atrophy of them and they were pale in color.

The peritoneal cavity was empty. All the abdominal organs were in normal position. Outside of a marked congestion they showed no abnormalities.

The level of the diaphragm was the fourth rib on both sides. The thymus was not

enlarged and was normal on cut section. There was about 25 c.c. of clear fluid in the pleural cavities.

Both lungs showed subpleural hemorrhages scattered over the surface of all lobes. The posterior portions of all lobes were airless and on section showed areas of collapse and of consolidation. The peribronchial lymph nodes were swollen and edematous.

The head was of normal size. The parietal eminences were somewhat prominent and the forehead flat. The anterior fontanelle was open, measuring 1 cm. transversely and 2 cm. longitudinally. The skull was normal. The external surface of the dura was normal.

There was a marked edema of the pia over the convexity of the cerebral hemispheres; the convolutions appeared normal. The general external configuration of the brain was normal. In the pia at the base, beginning at a point 1.5 cm. below the lower edge of the pons was a diffuse infiltration of the pia with recently shed, poorly clotted blood. The hemorrhages extended laterally about 0.5 cm. over the adjoining portions of the cerebellum.

Fig. 5. (Case 2.) Skeletal muscle.

Transverse cut sections through the brain and brain stem showed no gross lesions. The hypophysis was normal. The large venous sinuses at the base of the skull were normal.

The venous plexus around the cord was greatly congested. There was a marked edema of the loose connective tissue around the cord, especially in the lower portion of the spinal canal and a considerable collection of spinal fluid within the dura. The dura and pia on both sides of the cord were normal. The cervical and lumbar enlargements were well developed. Cross sections of the cord in representative regions showed no gross lesions.

Smears of the consolidated portions of the lungs showed many polymorphonuclear leucocytes and a great number of gram-positive diplococci.

Histologic examination of the kidney, liver, pancreas, thymus, peribronchial lymph glands, stomach, large and small intestine and spleen showed no abnormalities except congestion. Sections of the lung showed the alveolar spaces filled with exudate and leucocytes.

To summarize, the examination, other than that of the nervous system which will be given in detail later, showed a general atrophy of the skeletal muscles including the heart muscle, and a bronchopneumonia. Death was evidently due to the latter and to the circula-

tory failure, evidenced by the marked dilatation of the heart, the marked congestion of the venous system and the edema.

Examination of the Nervous and Muscular Systems.—Sections of striated muscle were examined from representatives groups, including the muscles of the leg, chest wall and abdomen and diaphragm. In all the sections the lesions were of the same degree and character, so that a single description will cover the pathologic lesions in all.

There is an almost complete disruption of all the muscle cells in every section and the various stages in the process of disintegration can be clearly followed. The least degree of damage consists in a swelling of the muscle cell to perhaps twice its normal size. The cross striation can still be made out. As the process becomes more severe these markings disappear and the cells take on a diffusely granular appearance. Vacuoles appear which with Sudan III are found to be filled with fat. This solution of the protoplasm of the cell allows structures to become visible which are not seen under normal conditions. This is particularly true of the sarcostyles of the muscle cell which are more resistant and therefore persist long after the remainder of the muscle cell is transformed into a granular mass. But

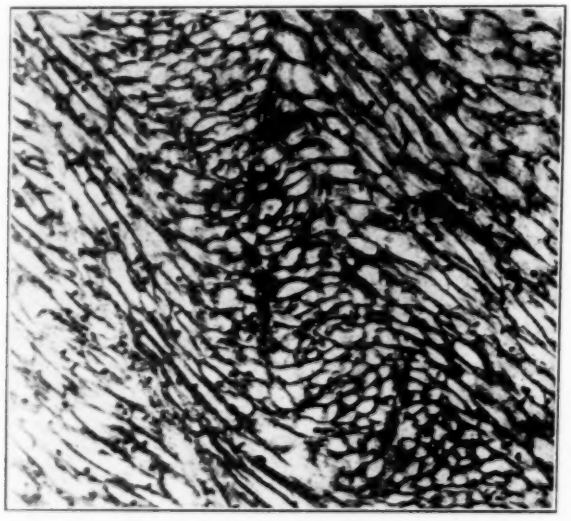

Fig. 6. (Case 2.) Cardiac muscle.

these structures also ultimately degenerate and the cell becomes a bag-like structure consisting of granular material and fat droplets contained in the intact sarcolemma (Fig. 5).

The changes noted in the cardiac muscle were equally severe, but of a somewhat different character. In them the change was principally a vacuolar one, all stages of the process from the appearance of a few small fat-containing droplets to complete transformation of the cell body into one large cavity, surrounded by a thin zone of granular protoplasm in which no fibrillae can be seen (Fig. 6). Practically every cell in sections from all parts of the heart were involved.

Sections from different parts of the nervous system were stained with the following methods: Van Gieson, hematoxylin and eosin, methylene blue, Giemsa, Weigert-Pal and Marchi.

The Peripheral Nerves.—No lesions could be seen with either Van Giesen's or hematoxylin and eosin stain in the peripheral nerves either from the brachial and lumbar plexus or in the finer ramifications contained in the sections of the muscle. With Marchi's method a slight degeneration was found in all those examined. Scattered between the nerve fibrils were a few small droplets from the degenerating myelin sheaths.

The Spinal Cord and Brain Stem.—The most extensive lesions found in the nervous

system were in the spinal cord and here the pathologic change was most marked in the motor cells. These cells present a most remarkable appearance. Throughout the entire cord all the motor cell groups and the cells of Clarke's column showed the same changes. These

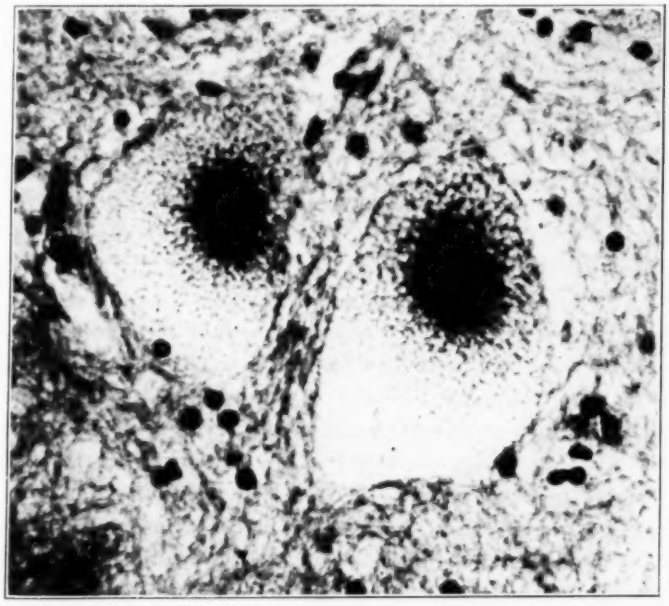

Fig. 7. (Case 2.) Anterior horn cells with approximately the same magnification as in Fig. 1.

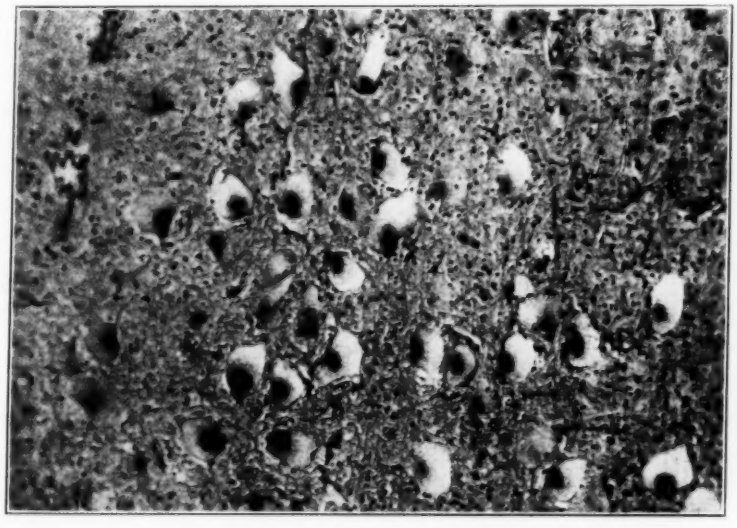

Fig. 8. (Case 2.) Group of anterior horn cells in lumbar region.

consisted of a marked swelling of the cell body, a solution of the tigroid material and eccentricity of the nucleus. The clear protoplasm consisted of a delicate network which became progressively more attentuated as the distance from the nucleus increased (Fig. 7). A group of these pale swollen cells from the lumbar region is shown in Fig. 8.

The same cell changes were also present in the nuclei of the medulla and brain stem.

The nuclei of all the cranial nerves, the olives and dentate nucleus of the cerebellum and the nuclei of the pons all showed the changes to an almost equal degree.

In the midbrain the same degeneration of the ganglion cells was also noted, though to an appreciably lesser degree, while in the cerebral cortex the pyramidal cells were entirely normal.

In sections stained by both the Marchi and Weigert-Pal methods for degeneration of the medulated fibers no definite lesions were found in any part of the central nervous system. Nor was there any evidence of proliferation of glia or of any inflammatory process such as perivascular round-cell infiltration or hemorrhages.

The pathologic changes in the nervous system can therefore be summarized as consisting of a widespread degeneration of the ganglion cells of the lower motor nerves with no evidence of tract degeneration or of any inflammatory process. The peripheral nerves are normal and there is an extensive degeneration of the skeletal muscle and the cardiac muscle. The degeneration in both the nervous and muscular systems is of the type commonly associated with acute processes.

Two points are worthy of comment in this case. In the first place the rapidity of the clinical course of the disease is in marked contrast to the protracted course, extending over several months or even years which has been observed in the majority of cases studied. The only similarly rapid case which we have found in the literature is one described by Balton, where the duration was three weeks. The anatomic changes also differ from those previously studied. In them the lesion is described as consisting of an atrophy, shrinkage and disappearance of the motor ganglion cells of the spinal cord, whereas, these cells in the present case show degenerative changes but of the so-called "acute" type, swelling of the cell, chromatolysis, irregularity and eccentricity of the nucleus. Our report therefore adds to the literature an acute case of progressive spinal muscular atrophy of children.

The second point of interest is the extensive involvement of the myocardial muscle.

SUMMARY

This paper is a clinical and pathologic report of two cases of early infantile progressive muscular atrophy of the Werdnig-Hoffmann type.

One case presents the usual pathologic findings of this disease consisting of marked atrophy of the anterior horn cells of the spinal cord and moderate chromatolysis of Clarke's column cells without evidence of an inflammatory process; the somatic muscles show extensive atrophic changes.

The second case is remarkable for the acute type of change in the anterior horn cells and the striking involvement of the heart muscle.

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THE ORGANIC PHOSPHORUS OF THE CEREBROSPINAL FLUID*

By Guy E. Youngburg, Ph.D., Buffalo, N. Y.

INTRODUCTION

WHILE the amount of inorganic phosphorus of cerebrospinal fluids has been rather definitely determined during the last several years and concordant and consistent results obtained by a number of different workers,[1, 2, 3, 4] the question of the occurrence and quantity of organically bound phosphorus has been given only slight attention.[2] The reasons for this are obvious, viz.: first, the small amount of organic phosphorus, and secondly, unsuitable analytic procedures.

Mestrezat,[5] Donath,[6] Apelt and Schumm,[7] Williamson,[8] and others, have found organic phosphorus. Haurowitz[2] concludes from his work that all of the phosphorus of the cerebrospinal fluid is in the inorganic form.

In consideration, first, of the possible clinical significance that might be attached to an increase or decrease of organic phosphorus through migration or degeneration of nerve tissue (phosphatides) of the central nervous system, or of products therefrom, or even phosphoproteins, in such disorders as tuberculous meningitis, neurosyphilis, lethargic encephalitis, etc., and secondly, in consideration of the now greatly improved micromethods for phosphorus determination, notably that of Benedict and Theis,[9] I have undertaken to obtain figures for the organic phosphorus of a large number of cerebrospinal fluids from patients with various disorders.

Although nerve tissue changes would be expected to be slow, yet it is not out of the question that such changes might be great enough to be reflected in the phosphorus content of the fluid.

Analyses for inorganic phosphorus were also made until it became evident that the content of inorganic phosphorus is entirely independent of the content of organic phosphorus, or vice versa.

ANALYTIC METHODS

Organic phosphorus was determined by precipitating inorganic phosphates with magnesia mixture, digesting the evaporated filtrate with sulphuric and nitric acids in the presence of a little copper sulphate and estimating the phosphorus by a modification of the Benedict and Theis[9] colorimetric method.

Special reagents used:

Magnesia mixture.—The widely used solution containing 55 gm. magnesium chloride, 70 gm. ammonium chloride and 88 c.c. of con. ammonia water per liter was employed.

*From the Biochemical Laboratories of the Buffalo City Hospital and the University of Buffalo Medical School.

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Ammonium molybdate.—3.1 per cent solution in water. The salt was purified by the Bell-Doisy[10] method.

Hydroquinone bisulphite.—0.5 gm. hydroquinone and 15 gm. sodium bisulphite per 100 c.c. of water solution.

Standard phosphate.—Solutions of KH_2PO_4, 5 c.c. equivalent to 0.0025 mg. and 0.005 mg. P.

Procedure.—Three c.c. of cerebrospinal fluid was transferred to a small test tube, 0.25 c.c. magnesia mixture was added, and after mixing, was allowed to stand from six to eighteen hours. The solution was then filtered into a 15 c.c. Pyrex test tube graduated at 10 c.c., the filter paper washed with several c.c. of water and the contents of the tube evaporated to dryness over a hot plate. 0.6 c.c. of sulphuric acid, 1 drop 10 per cent copper sulphate and 2 silica pebbles were then added and the tube was heated with a micro burner. When white fumes appeared a drop or two of dilute nitric acid (1 to 10) was added. If the contents did not decolorize upon heating again, more nitric acid was added. Heating was continued until all of the nitric acid was driven off, without at the same time losing any of the sulphuric fumes. After cooling, 5 c.c. of water, 2 c.c. of ammonium molybdate and 1 c.c. of hydroquinone-bisulphite were added, mixing after each addition, and the solution was made up to the mark and mixed.

Standards containing 0.0025 and 0.005 mg. P were heated to boiling with 0.6 c.c. sulphuric acid and 1 drop of copper sulphate as above and completed as for the unknowns. Color comparisons were then made in a colorimeter.

Blank determinations must give but a trace of blue.

Notes on above method: Magnesia mixture is widely used as a complete phosphate precipitant and it has been found in this laboratory[11] to be the most efficient for such a purpose as this.

While it would be desirable to develop more color, it is often difficult to obtain more than 3 c.c. of cerebrospinal fluid when quantities for routine tests must first be taken. The amount of color obtained by the Benedict and Theis procedure is, however, astonishingly great for a very small amount of phosphorus and the determinations reported were made with sufficient accuracy although they are only to be considered as approximate values.

The Benedict and Theis method was used for the determination of inorganic phosphorus.

EXPERIMENTAL

Cerebrospinal fluids were obtained as they came to the general laboratories of the hospital for the usual determinations. They were from both ward and out-patient departments and represent various disorders. None of the fluids used contained any visible traces of blood, although three showed red blood cells.

Globulin, cell count, colloidal gold and Wassermann tests were made as routine work and the phosphorus determinations as special work. The results on 200 fluids were obtained, but for the sake of brevity only the results of 20 typical ones are presented in Table I.

TABLE I

SHOWING ANALYSES OF 20 TYPICAL CEREBROSPINAL FLUIDS FROM HOSPITAL AND OUT-PATIENT CASES

NO.	GLOBULIN	CELLS	LANGE COLLOIDAL GOLD CURVE	WASSERMANN	ORGANIC P	INORGANIC P	REMARKS
		No. per c.mm.			Mg. per 100 c.c.	Mg. per 100 c.c.	
1	4+	210	Typical meningitis	?	0.59	4.19	
2	4+	116	?	++	0.23	1.07	
3	3+	32	Paretic	++	0.13	1.87	
4	3+	14	"	++	0.15	1.58	
5	3+	Too numerous	Meningitic	Anticomp.	0.41	1.35	A purulent fluid (pneumococcus).
6	2+	13	Luetic	++	0.11	1.35	
7	2+	33	"	++	0.29	1.27	
8	1+	9	—	++	0.12	1.51	Previous luetic gold curve.
9	1+	5	Weak luetic	-	0.17	0.96	
10	1+	169	Luetic	++	0.06	1.31	
11	1+	84	"	-	0.19	1.63	Acute encephalitis. Patient 13 years old.
12	1+	7	Paretic	++	0.20	0.97	
13	trace	4	—	-	0.14	1.33	
14	"	4	—	-	0.13	1.26	
15	"	12	Luetic	++	0.10	1.65	
16	-	1	—	-	0.13	1.26	
17	-	8	—	-	0.14	1.28	
18	-	1	Luetic	++	0.16	1.30	
19	-	3	—	++	0.18	1.38	
20	-	2	Luetic	-	0.21	1.35	

Surplus fluids were pooled and evaporated to dryness below 75° C. Five gm. of solids were extracted with 100 c.c. of warm alcohol-ether (redist. alcohol 3, redist. ether 1), filtered, digested with acid and phosphorus determined as indicated under analytic methods. The object was to determine lipoid phosphorus. Since this was found to be practically nil the phosphorus was probably present as a constituent of protein and the fluids have thus been arranged in the order of decreasing amounts of globulin.

Table II gives determinations of organic phosphorus on five postmortem fluids. This work was done to find if there is a rapid, slow, or no influx of organic phosphorus compounds after death, or if these compounds split off phosphoric acid readily.

TABLE II

SHOWING AMOUNT OF ORGANIC PHOSPHORUS IN POSTMORTEM CEREBROSPINAL FLUIDS

NO.	HOURS AFTER DEATH	ORGANIC P MG. PER 100 C.C.	PATHOLOGY
1	1	0.10	Pulmonary tuberculosis.
2	4	0.15	" "
3	8	0.29	Chronic cardiac decomposition.
4	9	0.12	Myocarditis.
5	10	0.07	Cardiorenal.

DISCUSSION

As shown in Table I (results on 20 typical fluids), the amount of organically bound phosphorus is very small, the maximum amount found in the examination of 200 fluids being 0.59 mg. and the minimum 0.06 mg. per cent. Only five fluids contained more than 0.4 mg. per cent. Two of these showed a meningitic colloidal gold curve, one showed a luetic curve, one showed a negative colloidal gold curve, and one fluid was not recorded for this test.

The variation in absolute amount of organic phosphorus is very small and no relation to the pathology can be pointed out except that on the average those fluids containing more globulin and cells and which gave a positive Wassermann test showed most organic phosphorus. But even in this respect there were a number of exceptions.

Since practically no lipoid phosphorus was found in the cerebrospinal fluid solids, the phosphorus is bound in some other form, the possibilities being, according to the present knowledge of phosphorus compounds of the body, a hexosephosphoric ester (Emden), a nucleotide (Jackson) or inosinic acid (Greenwald). At any rate, *it is impossible to point out any nerve-tissue changes by the amount of organic phosphorus.* This was the point in particular on which data were desired.

It appears from Table II that the change in the amount of organic phosphorus after death is very slow.

It can be seen from Table I that the inorganic and organic phosphorus are entirely independent of each other, e.g., fluids with high values for organic phosphorus may have high or low inorganic phosphorus content.

SUMMARY

Two hundred cerebrospinal fluids, from hospital and out-patient cases, have been analyzed particularly as to content of organically bound phosphorus. Between 0.06 mg. and 0.59 mg. per cent of phosphorus was found, the great majority being between 0.1 mg. and 0.3 mg. per cent. This phosphorus is not in the form of lipoids but in all probability in protein combination.

No diagnostic value can yet be attached to the determination of organic phosphorus.

Organic and inorganic phosphorus contents are independent of each other.

Thanks are due Miss Marjorie Bauckus of the serologic laboratory for help in obtaining the fluids.

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A STUDY OF THE TOXIC FILTRATES OF ANHEMOLYTIC STREPTOCOCCI, RECOVERED FROM PATIENTS WITH RHEUMATIC FEVER*

By Edward Steinfield, M.D., and Maurice S. Jacobs, M.D.
Philadelphia, Pa.

THE literature of rheumatic fever contains numerous researches dealing with the relation of streptococci to this disease. Probably the most exhaustive is the well-known contribution of Poynton and Paine.[1] Other investigators have endeavored to adduce evidence in favor of the streptococcic origin of rheumatic fever and rheumatic endocarditis, or have indicated such relationship by the demonstration of immune reactions in patients with this disease. Among the early workers, Triboulet and Coyon[2] implicated a diplococcus found in the blood of rheumatic fever patients; Westphal, Wassermann and Malkoff[3] cultivated from the heart's blood in fatal cases, a streptococcus which grew in mediums of high alkalinity. Tunnicliff[4] found that opsonins for streptococci were increased in the blood of patients with acute rheumatic fever and in seven out of twelve cases, agglutinins were demonstrated. Kinsella and Swift,[5] however, were unable to demonstrate that strains of anhemolytic streptococci recovered from patients with rheumatic fever belonged in the same biochemical or immunologic group. Derick and Andrewes[6] have noted differences between strains of anhemolytic streptococci derived from throat cultures of patients with rheumatic fever and cultures from normal individuals. The former gave skin reactions in rabbits with a secondary reaction nine days later; this did not occur with anhemolytic streptococci from normal throats. Interesting observations have been recorded by Miller[7] in attempts to transmit rheumatic fever to rabbits and guinea pigs with various materials from patients with this disease, supposedly containing the infective agent. He used (1) blood (usually uncoagulated) taken from the vein during acute stages of rheumatic fever; (2) joint fluid aspirated from the involved joints and anaerobic cultures from the joint fluid; (3) pleural fluid; (4) throat washings which had been passed through a Berkefeld N filter; (5) extracts of tonsillar tissue. These inocula were injected into twenty-seven young rabbits and fourteen young guinea pigs. A definite arthritis occurred in only one rabbit and one guinea pig. The guinea pig had been directly inoculated from the throat washings of a patient. The rabbit, however, had been injected with the blood and suspension of heart muscle from another rabbit which had received the whole blood from a patient with rheumatic fever. Though these two observations may be said to be isolated instances, they are suggestive, as Miller points out, because of the unlikelihood of spontaneous arthritis in rabbits and guinea pigs.

*From the Laboratories of the Jewish Hospital.
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Among the cogent arguments against the streptococcic etiology of rheumatic endocarditis and myocarditis is the inability to produce true Aschoff nodules in the animal after injection with anhemolytic streptococci. Topley and Weir[8] inoculated twenty-nine rabbits with cultures of streptococci isolated at necropsy from the mitral valve of a case of rheumatic endocarditis. Of these, twenty-six developed fever; twenty-three developed arthritis and two developed endocarditis. The infiltrations in the heart, however, resembled Bracht-Wächter lesions rather than the characteristic submiliary nodules described by Aschoff. Thalhimer and Rothschild,[9] and later Cecil[10] described the distinguishing features in the hearts of animals injected with streptococci and indicated their resemblance to lesions of subacute bacterial endocarditis in contrast to those of rheumatic fever. The possible error due to infiltrations produced by foreign protein has been pointed out by Longcope[11] who described collections of round cells in the heart muscle of rabbits after injection of egg albumen and horse serum. Another factor has been noted by Miller[12] in the spontaneous interstitial myocarditis found in apparently normal rabbits.

Blood cultures taken in the course of rheumatic fever are frequently sterile in contradistinction to the high percentage of positive blood cultures in subacute bacterial endocarditis. Recently, Clawson[13] has described observations in which strains of Streptococcus viridans were isolated from twenty cases of well-defined acute rheumatic fever, rheumatic endocarditis or chorea of which thirteen were derived from blood cultures during life. The high percentage of positive blood cultures is attributed by him to the following technic: Fifty c.c. of blood were collected in two test tubes and allowed to clot; the clots are later loosened and put into flasks of 250 c.c. of dextrose beef infusion. Of twelve strains tested, nine produced endocarditis in animals with apparently typical vegetations and the organisms were recovered in the heart's blood and joint. Agglutinins for the streptococci were found in four out of five cases of rheumatic fever in dilution of 1:50 or more.

Though our own studies are concerned with a toxic principle derived from cultures of anhemolytic streptococci, presumably an exotoxin, the work of Herry[14] upon an endotoxin from similar strains, is somewhat applicable. Since most of these strains, however, were obtained from blood cultures, it suggests the possibility that some of these patients may have suffered from subacute bacterial endocarditis. After grinding sediments of these cultures with salt and resuspending in distilled water for twenty-four hours, he used the clear supernatant fluid filtered through a Chamberland filter. This material produced death in rabbits in five to twelve days. He described lesions in the heart muscle which were thought to resemble Aschoff bodies.

The present report is based upon the toxic effects of Berkefeld filtrates from cultures of anhemolytic streptococci, derived from throat cultures of patients with rheumatic fever or rheumatic endocarditis. For the purpose of comparison similar strains from normal individuals and one strain isolated from blood cultures of a patient with subacute bacterial endocarditis, were also used.

METHODS

Cultures of anhemolytic streptococci were obtained from eight sources. Five were from throat cultures of patients with rheumatic endocarditis; one was from a positive blood culture in the course of subacute bacterial endocarditis; two were obtained from throat cultures of normal individuals. Cultures from the throat were made on Loeffler's blood serum and also streaked on blood agar plates. Transplants from colonies of anhemolytic streptococci, usually green producing, were made in flasks of blood-dextrose bouillon and in bottles of Loeffler's media containing considerable condensation fluid. All the strains studied fermented lactose and salicin, but not mannitol. After incubation for two to three days, the sediment was removed by centrifugation, the supernatant fluid diluted with an equal volume of normal saline and filtered through Berkefeld N filters. Varying speeds of filtration were used but no apparent difference in toxicity due to this cause, was noted. The filtrates were used as inocula. Filtrates which had been heated for varying periods in the Arnold sterilizer and in the autoclave under 15 pounds pressure were also studied. Guinea pigs were inoculated by intramuscular and intraperitoneal injections. Intracardiac methods were tried but found uncertain and not suitable because of the resulting interference with histologic examination.

PROTOCOLS OF RHEUMATIC FEVER PATIENTS

1. *Acute Rheumatic Fever.*—H. G., male, aged forty-four, had mild attack of joint pains three years prior to the present observation, at which time he developed polyarthritis. Associated with this he had fever varying between 99° F. and 101.6° F. The heart at no time was affected. The tonsils were diseased and cultures produced a profuse growth of Streptococcus viridans. Roentgenograms of the teeth revealed periapical infection.

2. *Rheumatic Endocarditis and Pericarditis.*—E. L., male, aged seventeen years. First attack of rheumatic fever three years before present observation at which time he developed endocarditis. Recurrence at present observation with fever, pericarditis and mitral presystolic and systolic murmurs. Heart enlarged to right and left with left border to the sixth rib in the anterior axillary line. P-R interval 0.14 second.

3. *Subacute Bacterial Endocarditis.*—H. L., male, aged seventy-two years. Chills and fever two months before admission, died one month after admission. Irregular, remittent fever, as high as 104° F. while in hospital. Apex beat fifth interspace just outside midclavicular line; a harsh mitral systolic murmur. Spleen was not palpable but liver was enlarged. No petechiae and no clubbing of fingers were noted. Blood count showed 2,910,000 red blood cells, hemoglobin 56 per cent, leucocytes 14,000. The urine showed a heavy cloud of albumin, red blood cells and granular casts. Blood cultures on two trials were positive for Streptococcus viridans. Necropsy revealed typical luxuriant and friable vegetations of the mitral valve extending up into the left auricle.

4. J. F., male, aged thirty-one, was admitted to hospital complaining of shifting pains and swelling in large joints of two weeks' duration. No previous attacks. Large diseased tonsils were present; a loud systolic murmur was heard at the mitral area transmitted to the axilla. Anhemolytic streptococci were found on throat culture. Temperature ranged between 98° F. and 100° F. Electrocardiogram revealed widening of the P-wave to 0.1 second with normal P-R interval. Marked improvement was noted in seven weeks; the murmur was not heard at the final examination.

5. *Acute Rheumatic Fever and Endocarditis.*—J. G., boy, aged thirteen years. Onset with sore throat and fever followed one week later by arthritis of both knees; rough systolic murmur at aortic area and slight enlargement of heart to left.

6. *Rheumatic Fever and Endocarditis Following Tonsillitis.*—J. C., male, twenty-six years of age, developed polyarthritis two weeks after an attack of tonsillitis. Ankles, knees, hips and shoulders were successively involved. Urticaria present. Diseased tonsils, enlargement of the heart and mitral presystolic and systolic murmurs were noted.

RESULTS

Thirty-four guinea pigs averaging 300 gm. in weight, were injected with the Berkefeld filtrates of cultures of anhemolytic streptococci recovered from the throats of five patients with rheumatic fever and endocarditis with sterile blood cultures, and from the blood of a patient with subacute bacterial endocarditis. Intramuscular and intraperitoneal injections were made in doses of 2 to 5 c.c. (representing 1 to 2.5 c.c. of undiluted filtrate). Twenty-three of the animals died in periods varying from one to twenty-one days. Deterioration was noted in all the cultures studied and after a number of transplantations, the lethal effect was gradually lost. The details are more clearly brought out in the table. Attempts to modify the toxic principle by heat

TABLE I

EFFECT OF INJECTION OF STREPTOCOCCIC FILTRATES INTO GUINEA PIGS

ANIMAL NO.	CULTURE	FILTRATE AMT. C.C.*		METHOD OF INOCULATION	RESULT
		Unheated	Heated		
1	H. G.	3 c.c.		Intramuscular	Died 21 days
2	"	4 c.c.		"	Died 18 "
3	E. L.	3 c.c.		"	Died 14 "
4	"	4 c.c.		"	Died 12 "
5	"	3 c.c.		"	Died 16 "
6	"	2 c.c.		"	Survived
7	"	2 c.c.		"	Died 19 "
8	"	3 c.c.		"	Died 15 "
9	"	5 c.c.		Intraperitoneal	Died 5 "
10	"	5 c.c.		"	Died 11 "
11	"	4 c.c.		"	Died 19 "
12	"	4 c.c. plus 1 c.c. serum of E. L.		Intramuscular	Died 4 "
13	"	4 c.c. plus 1 c.c. control serum		"	Died 4 "
14	"		90° C. 1 hr. 3 c.c.	"	Died 9 "
15	"		100° C. 1 hr. 3 c.c.	"	Died 22 "
16	H. L.	1 c.c.		Intraperitoneal	Died 6 "
17	"	2 c.c.		"	Died 4 "
18	"	3 c.c.		"	Died 3 "
19	"	4 c.c.		"	Died 24 hours
20	"	3 c.c.		"	Died 13 days
21	"	5 c.c.		"	Died 10 "
22	"†	5 c.c.		"	Survived
23	"	5 c.c.		"	Survived
24	J. F.	2 c.c.		"	Survived
25	"	5 c.c.		"	Survived
26	J. G.	3 c.c.		"	Died 1 day
27	"	2 c.c.		"	Died 3 days
28	"	1 c.c.		"	Died 9 "
29	H. T. (normal)	4 c.c.		"	Survived
30	"	5 c.c.		"	Survived
31	E. S. (normal)	4 c.c.		"	Survived
32	"	5 c.c.		"	Survived
33	J. C.	4 c.c.		"	Survived
34	"	5 c.c.		"	Survived

*Filtrate represents 1:2 dilution of supernatant fluid of culture media.
†Culture in fifth transplantation.

less than 100° C., or by the addition of normal serum or serum from patients with rheumatic fever, appeared to enhance the toxicity. In all instances several days before death the heart rate of the animal was considerably lowered, frequently falling from a normal rate of 180 to 200 beats per minute down to 100 beats per minute, as noted by auscultation. A loss in weight was also observed if the animal survived over a week. No instance of arthritis was noted. At necropsy, cultures from the heart's blood were sterile. Firm clots were found in the heart cavity but no discernible lesion of the valves was noted, though obviously conclusions on gross inspection of these structures were invalidated by their small dimensions. Microscopic section and staining with hematoxylin and eosin revealed no areas resembling Aschoff bodies. Selective staining with the Unna-Pappenheim method was not done because nothing suggesting submiliary collection of plasma cells was noted. A slight cloudy swelling of the myocardium was found in some of the specimens. The toxic principle was apparently not produced regularly by all strains found in throat cultures from rheumatic patients. In similar attempts in two other cases, entirely negative results were obtained. Negative results were also noted with the strains from two normal individuals.

DISCUSSION

The question as to whether we were dealing with a true exotoxin is not definitely determined. Experiments in producing an antitoxin capable of protecting the animal in multiple proportions, were only partially carried out due to the large doses of filtrate necessary to establish this point. The toxic property is rather small when the comparatively large dose is considered. Its marked resistance to heat creates a resemblance to an endotoxin though the same property is manifested by Dick's scarlatinal toxin. The definite though variable incubation period regularly observed is however more often noted with a true exotoxin. The experiments of Herry indicated similar incubation periods with the use of material presumably containing an endotoxin.

SUMMARY

1. Berkefeld filtrates of cultures of anhemolytic streptococci isolated from the throats (particularly the tonsils) of three patients with rheumatic fever were lethal for guinea pigs in doses of 2 to 4 c.c. (representing 1 to 2 c.c. of original culture fluid). This property was also noted in the cultures of anhemolytic streptococci isolated from the blood of a patient with subacute bacterial endocarditis. Two other strains isolated from patients with rheumatic fever and two from normal individuals did not produce toxic filtrates.

2. The action of the toxic filtrate was evidenced by a slowing of the heart rate in guinea pigs and death in one to two weeks with some strains and in one to nine days with other strains. Deterioration was noted after a number of transplants with a loss in lethal power. Aschoff nodules and arthritis were not produced in the experimental animals.

3. The toxic principle, when present, was relatively weak in view of the comparatively large doses which were used.

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THE ENDOTHELIOID CELL IN ACUTE LEUCEMIA*

By R. B. Barton, M.D., Boston, Mass.

THE significance of endothelial and endothelioid cells in the leucemias has been the basis of considerable speculation with very little substantial experimental background. It is known that in chronic myeloid leucemia the absolute endothelial count is increased, this usually being ascribed to a concomitant reaction of the endothelial system to the leucemic stimulus. Further, a third classification of leucemias,—the endothelial cell leucemias,—has been made. The latter, as reported, have all been more or less acute in course, clinically resembling acute leucemia of either conventional type.

Undoubtedly, the so-called endothelial cell leucemia is a rare condition, although in all probability not so rare as the literature indicates. The cases that have been reported are noteworthy for the paucity of pathologic detail they present with but one outstanding exception. Ewald† has reported a case of acute leucemia, with a rapidly fatal course, in which the predominant cells were distinctly endothelioid,—similar, indeed, in every respect to those seen in the case outlined below. He prepared excellent sections which showed definite extracapillary budding of the atypical cell within the bone marrow. This would seem to give it a place in the leucocyte series, disregarding the obvious morphologic evidence; in fact, Ewald hinted that it might be an earlier form of myeloblast although he eventually classified it as erythroblast. The Ewald case and the extraordinarily fine histopathologic preparations for which Ewald is responsible go far toward substantiation of the conclusions drawn from the following case.

CASE HISTORY

On February 16, 1926, a man thirty-six years old was admitted to the Haynes Memorial Hospital complaining of asthenia and vertigo of about ten days' duration. The referring physician suspected diphtheria as there was a heavy yellow-white membrane on the hard palate and the left tonsil. There were marked gastrointestinal symptoms,—nausea, vomiting and diarrhea with tarry stools. The patient was obviously very ill, being dyspneic and cyanotic,

*From the Evans Memorial.
†Ewald, Otto: Deutches Arch. f. klin. Med., 1922, cxlii, 222.

and tossing restlessly in bed. His lips were pale, the mouth dry and incrusted with sordes, and the facial color was gray with a distinctly yellowish cast. Examination revealed little further. The heart was slightly enlarged, and there were loud murmurs of the hemic type over all the valve areas. The abdomen was negative. A neurologic examination, which was done when a question of pernicious anemia arose, was essentially negative. There was no demonstrable adenopathy, and the spleen was not palpable. The patient remained in the hospital seven days with a temperature oscillating between 101.2 and 102.4, and died on the morning of the eighth day with pulmonary edema and myocardial failure. The only therapy given consisted of a prophylactic dose of 30,000 units of diphtheria antitoxin on admission, and a liquid diet. Smears prepared from the membrane on the tonsil showed a definite Vincent's angina. Necropsy was performed about five hours postmortem.

Gross examination of the tissues showed nothing more than a pulmonary edema and dilated heart. The cardiac tissue was of the "tiger lily" type, showing marked fatty degeneration. There was no adenopathy beyond a very slight enlargement of the mesenteric glands, which were removed for section. Microscopic examination of spleen, liver, bone marrow and lymph nodes showed typical leucemic infiltration in which the atypical cell of the circulating blood was predominant. The bone marrow, which was pink and liquescent in the gross, was taken from the shaft of the femur. Unfortunately, it was not possible to demonstrate the direct proliferation of the cells from the bone marrow capillaries in a satisfactory manner.

The day following admission of the patient a blood count was done by the interne. A red count of 1,370,000 and a white of 2,000 was reported. The differential count showed 80 per cent of "lymphoid" cells. Daily counts thereafter showed a tendency to lowering of the white count, which dropped to 1,300 on the third day. On February 27, the blood was examined in the Evans Memorial laboratories, the question of pernicious anemia having arisen. At this time a red count of 1,350,000 and a leucocyte count of 3,650, of which 57.8 per cent were myeloblasts, was obtained. The myeloblasts were entirely characteristic, most of them yielding a peroxidase reaction with the Goodpasture stain. The following morning, five hours before the patient's death, a second count was made. The red count was very slightly increased, while the leucocyte count had risen to a distinctly leucemic value of 29,950. Coincident with this rather remarkable change, the character of the predominant cell was altered, the myeloblasts which here formed only 23.5 per cent of the differential count, having been replaced by a cell classified as endothelioid which formed 46 per cent of the total count.

The endothelioid cell, which could not be sharply differentiated from the myeloblast in all cases, showed the following characteristics: The diameter varied from 10 to 40 microns, the average being about 20 microns. The outline was irregular, in some cases showing a tendency toward separation of cytoplasmic fragments, strongly suggestive of the megakaryocyte. The nucleus was multilobulated, presenting a fine granular appearance characteristic of many early cells. There were present from one to four nucleoli or plasmosomes. In not a few instances the cell was polynuclear. The cytoplasm stained deeply basophilic and somewhat unevenly, a perinuclear zone being almost unstained with both Wright's and Giemsa's stains. The granules were irregular in size, azurophilic to slightly basophilic and showed a tendency to grouping and peripheral distribution. The cells in many instances yielded a marked peroxidase granulation with the Goodpasture stain. As has been intimated, the morphology varied considerably, ranging from a type undoubtedly endothelial to one distinctly myeloblastic. The leucemia was complicated by a marked anemia with many primary characteristics and a tendency to aplasia.

It is believed that the case above should be diagnosed as a premyeloblastic or endotheloid, rather than an endothelial cell leucemia as had been suggested. The evidence to support this contention is found both in the case reported above and in the Ewald case. The case described, on admission was distinctly that of aleucemic leucemia evidently of a rapidly progressive type. This was accompanied by an anemia which was somewhat aplastic. In view of these two conditions the bone marrow may be said to have become exhausted or to have lost its power to a large extent, of both erythro- and leucogenesis. A few

TABLE I

BLOOD COUNTS

SEPT. 22				SEPT. 23
Erythrocytes		1,350,000		1,550,000
Leucocytes		3,650		29,950
Hemoglobin		30%		35%
Color index		1.1		1.1
Platelets		130,000		280,000
		Differential Count		
Cells counted		500		200
Lymphocytes, small	18%		16.5	
large	4.6%	22.6	0.5	17.0
Plasma cells		0.2		1.5
Mast "		0.2		0
Eosinophiles		0		0
Polymorphonuclears		5.4		5.5
Endothelial	1. mono. 0.8			
	trans. 2.6	3.4		0
Lymphoblasts		0.2		0
Basophilic myelocytes		0		0
Acidophilic "		0		0
Neutrophilic		4.8		1.5
Myeloblasts		57.8		45.5
Endothelioid cells		0.6		49.0
Megakaryocytes		0.6		0
Erythroblasts		0		0.5
		In Above Differential Counts		
Anisocytosis	Marked, many megalocytes in both			
Poikilocytosis	Marked, "primary" type in both			
Polychromasia	Definite			
Normoblasts	3			7
	No micro- or megaloblasts.			

hours before death the leucemia changed to the leucemic form, thus theoretically thrusting upon the exhausted bone marrow, already incapable of producing more than an extremely immature cell, a tremendous strain. This is believed to have provoked the production of a cell earlier in the myelogenous series than the myeloblasts and in all probability the parent cell of the myeloblast. It is also possible that the endothelioid cell represents an abortive form not identical with any of the developmental forms in a myeloid series. In any event, it indubitably represents an earlier form than has been hitherto recognized. To substantiate these deductions Ewald's demonstration of the direct extracapillary proliferation of the endothelial cells of the bone marrow capillaries seems to give the lacking histopathologic evidence.

The findings above are not entirely unique. The endothelioid cell has been further observed in far smaller numbers in two cases of rapidly fatal acute myelogenous leucemia, and at the height of an exacerbation of a chronic myelogenous leucemia in which myeloblasts were numerous. It is suggested that it may yield some prognostic significance.

In conclusion, then, there is both direct and indirect evidence that there is present in the circulating blood under certain unusual conditions a cell with distinctly endothelial characteristics intermediate between the endothelial cell of the bone marrow capillaries and the myeloblast of acute leucemias.

THE DIAGNOSTIC VALUE OF THE SUGAR CONTENT IN THE CEREBROSPINAL FLUID*

By Alfred S. Giordano, M.D., M.Sc., South Bend, Indiana†

THE intensive study during the last few years of the chemical composition of the cerebrospinal fluid has yielded a great deal of valuable information to aid in the differential diagnosis of certain obscure cases of brain tumor, encephalitis, poliomyelitis and various forms of meningitis. It is my purpose in this report to emphasize the value and the limitation of the quantitative determination of sugar in the differential diagnosis of such conditions with special reference to tuberculous meningitis.

In cases of pyogenic meningitis and tuberculous meningitis the sugar content of the cerebrospinal fluid is either zero or markedly diminished. In cases of poliomyelitis, encephalitis and other nonpurulent affections of the central nervous system the glucose content is extremely variable (Table I). Some authors, notably Foster and Cookson, have advanced the theory that the sugar content of the spinal fluid in encephalitis is relatively higher than in poliomyelitis. This conclusion is not generally accepted. At present there still exists some confusion as to the range of sugar in the normal spinal fluid.

I shall report additional data on this question based on a series of 127 examinations of spinal fluid; thirty-one were made in twenty-one cases of tuberculous meningitis and the remaining comprise a group of various normal and pathologic controls.

METHOD

The Folin and Wu method, with precipitation of the proteins by the tungstic acid reagents, was used for the quantitative sugar determinations. When the sugar content was below 25 mg. double amounts of filtrate were used or the dilution of the unknown was made up to 12.5 c.c. instead of 25 c.c., thus facilitating the reading. All nonpyogenic spinal fluids with a sugar content of less than 70 mg. were inoculated into guinea pigs to determine the presence or absence of bacilli of tuberculosis. All globulin estimations were made by the Noguchi method using 0.2, 0.5 and 0.1 c.c.

DIAGNOSIS

The diagnosis of tuberculous meningitis in all cases reported was corroborated either by necropsy, by guinea pig inoculation of the spinal fluid or by both, and in a few instances the bacilli of tuberculosis were demonstrated in smears. This method, however, was not depended on for obvious reasons. In the cases of pyogenic meningitis the diagnosis was proved by cultures and

*Read before the resident and ex-resident physicians of Mayo Clinic, October, 1926.
†From the South Bend Medical Laboratory.
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TABLE I

COMPARATIVE REPORTS FROM THE LITERATURE

AUTHORS	NORMAL			VARIOUS NEUROLOGIC AFFECTIONS			NEUROSYPHILIS			ENCEPHALITIS			POLIOMYELITIS			PURULENT MENINGITIS			TUBERCULOUS MENINGITIS		
	CASES	RANGE	AVERAGE	CASES	RANGE	AVERAGE	CASES	RANGE	AVERAGE	CASES	RANGE	AVERAGE	CASES	RANGE	AVERAGE	CASES	RANGE	AVERAGE	CASES	RANGE	AVERAGE
Wilcox and Lyttle	23	48 to 100	60	21	50 to 120	80	3	20 to 100	50	14	30 to 100	73	4	45 to 99	66	9	0 to 91	34	24	0 to 45	25
Stowe	10	60 to 90	83	14	70 to 114	90	15	60 to 91	77	20	60 to 182	81	..	..	..	14	0 to 30	..	21	10 to 50	30
Giordano	33	60 to 90	73	25	47 to 136	107	20	50 to 88	68.5	5	81 to 183	109	3	62 to 94	77	6	0 to 40	17.5	21	0 to 60	22.3
Levinson	5	60 to 73	65	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	10	11 to 35	24
Spurling and Maddock	..	57 to 84	74	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Foster	11	40 to 60	76	..	..	..	..	..	..	39	50 to 113	74	..	..	..	5	0 to 45	70	11	0.6 to 41	25
McLean and Von Hofe	..	45 to 85	..	..	..	..	..	..	..	..	..	..	..	..	..	35	0 to 45	..	..	..	28
Kubie and Shults	..	.	..	..	53 to 81	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

smears. The complement-fixation test (Kolmer) was positive in all patients having syphilis. The presence of brain tumor was proved at necropsy. The other diagnoses were submitted by the clinical consultants.

RESULTS

The results of this study are shown in Tables II to VII. Table II contains a group of thirty-three apparently normal control specimens consisting chiefly of spinal fluids taken from patients under treatment for syphilis and from patients with other diseases probably not involving the central nervous system. The range of the sugar content is from 60 to 90 mg. These figures closely parallel those of Stowe, Spurling and Maddock and are much higher than the earlier reports by the French workers and by Foster.

TABLE II

NORMAL CONTROLS

CASES	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN, GRADE	SUGAR MG.	GUINEA PIG INOCULATION
1	Suspected syphilis of the central nervous system	0	0	77	
2	Pulmonary tuberculosis	0	0	70	
3	Sinusitis	0	2+	66	Negative
4	Carcinoma of prostate	0	0	70	
5	Mercurial poisoning	6	0	75	
6	Pneumonia	0	1+	83	
7	Otitis media	0	0	68	
8	Syphilis	0	0	90	
9	Syphilis	0	0	80	
10	Syphilis	0	0	80	
11	Syphilis	0	0	75	
12	Syphilis	0	0	72	
13	Syphilis	0	0	71	
14	Syphilis	0	0	68	
15	Syphilis	0	1+	81	
16	Syphilis	0	0	77	
17	Syphilis	0	0	70	
18	Syphilis	0	0	73	
19	Syphilis	0	0	82	
20	Syphilis	0	0	65	Negative
21	Syphilis	0	0	66	Negative
22	Syphilis	0	0	73	
23	Syphilis	0	0	72	
24	Syphilis	0	0	71	
25	Syphilis	0	0	82	
26	Syphilis	0	0	65	Negative
27	Syphilis	0	0	72	
28	Syphilis	0	0	74	
29	Syphilis	0	0	67	Negative
30	Syphilis	5	0	79	
31	Syphilis	0	0	73	
32	Syphilis	0	1+	60	Negative
33	Syphilis	0	0	78	

The group including the various neurologic conditions (Table III), is of significance since the range of the sugar content is between 47 and 136 mg. with an average of 107 mg. Cases 2, 3 and 4 technically should be classified as encephalitis if the term is used in the general rather than in the restricted sense to cover cases of epidemic encephalitis lethargica. Case 11 is of particular in-

TABLE III

VARIOUS NEUROLOGIC AFFECTIONS

CASES	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN, GRADE	SUGAR MG.	GUINEA PIG INOCULATION
1	Brain abscess	0	0	71.8	
2	Acidosis (acute enteritis)	20	4+	109.0	
3	Uremia (nephritis)	6	1+	136.0	
4	Meningism (pyelitis)	0	0	110.0	
5	Meningism (typhoid fever)	4	0	71.0	
6	Meningism (enteritis)	5	1+	70.6	
7	Meningism (enteritis)	1	0	60.5	Negative
8	Cerebral hemorrhage	30	1+	61.0	Negative
9	Cerebral hemorrhage	0	0	80.0	
10	Cerebral hemorrhage	*	1+	90.0	
11	Cerebral hemorrhage	3170	1+	52.0	Negative
		150**	0	47.0	Negative
12	Intracranial hemorrhage	22	4+	80.0	
13	Brain tumor	0	0	65.0	Negative
14	Brain tumor	5	0	72.0	
15	Tetanus	3	0	77.0	
16	Tetanus	0	0	83.0	
17	Traumatic injury of head	0	0	90.0	
18	Epilepsy	0	0	70.0	
19	Epilepsy	6	0	86.0	
20	Psychoneurosis	0	0	86.0	
21	Psychoneurosis	0	0	88.0	
22	Manic depression	0	0	90.0	
23	Hydrocephalus	40	4+	75.0	
24	Neuritis	160	4+	106.0	

*Red blood cells.

**Four days later.

TABLE IV

SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

CASES	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN, GRADE	SUGAR MG.	WASSERMANN TEST (KOLMER)
1	Gastric crisis	50	2+	82	4, 4, 3, –
2	Tabes dorsalis	20	1+	70	4, 4, 4, –
3	Tabes dorsalis		2+	75	4, 4, 4, –
4	Charcot joint	130	4+	50	4, 4, 4, –
5	Juvenile tabes	60	3+	63	4, 4, 4, 4
		20	3+	52	4, 4, 4, 4
6	Paresis	77	–	66	4, 3, 1, –
7	Tabes dorsalis	10	1+	66	4, 4, –, –
8	Paresis	25	3+	60	4, 4, 4, –
9	Paresis	68	4+	74	4, 4, 4, –
10	Paresis	8	2+	67	4, 4, 4, 4
11	Tabes dorsalis	45	4+	63	4, 4, 4, –
12	Syphilis (treated)	8	1+	70	2, 2, 1, –
13	Wrist-drop	35	2+	75	2, 2, 2, –
14	Gastric crisis	25	1+	85	2, 1, –, –
15	Tabes dorsalis	80	1+	65	4, 4, 4, 3
16	Tabes dorsalis	61	1+	88	4, 4, –, –
17	Tabes dorsalis	110	4+	58	4, 4, 4, 3
18	Suspected syphilis	100	1+	71	4, 4, –, –
19	Paresis	10	1+	70	2, 1, –, –

terest since the sugar content is within the range of tuberculous meningitis; it also has all the other characteristics, clear spinal fluid with a high cell count (3,170 for each c.mm.), 1+ globulin, negative culture and no bacteria demon-

TABLE V

PURULENT MENINGITIS

CASE	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN, GRADE	SUGAR, MG.	ORGANISM FOUND ON CULTURE	ORGANISM FOUND IN SMEAR
1	Scarlet fever meningitis	350	4+	40	Streptococcus hemolyticus	Streptococcus
2	Influenza meningitis	2400	4+	0	None	Bacillus influenzae
3	Ulcerative endocarditis	700	4+	15	Streptococcus viridans	Streptococcus
4	Infected hydrocephalus	170	4+	35	Staphylococcus	Staphylococcus
5	Epidemic meningitis	2400	4+	5	Gram-negative diplococcus	Meningococcus
6	Pneumococcal meningitis	2000	4+	0	Pneumococcus	Pneumococcus
7	Otitis media meningitis	1200	3+	0	Streptococcus viridans	Streptococcus
8	Otitis media meningitis	4000	4+	0	Streptococcus hemolyticus	Streptococcus
9	Meningitis	2300	4+	10	Streptococcus pyogenes	Streptococcus
1	Poliomyelitis	92	3+	62	None	None
2	Poliomyelitis	94	2+	94	None	None
3	Poliomyelitis	22	4+	77	None	None
4	Encephalitis	0	1+	81	None	None
5	Encephalitis	15	–	84	None	None
6	Encephalitis	0	2+	110	None	None
7	Encephalitis	0	0	90	None	None
		15*	0	183	None	None

*One hour before death.

strable in the smear. The clinical findings, however, supported the presumptive diagnosis of cerebral hemorrhage or thrombosis and were further corroborated by the subsequent improvement of the patient and the negative result of the guinea pig inoculation for bacilli of tuberculosis. This is the only instance in this series of cases in which a diagnosis of tuberculous meningitis based on the low sugar content would have been erroneous. But is it sufficient to demonstrate the need of additional data on the subject.

The group of patients having neurosyphilis (Table IV) is easily distinguished from those having meningitis because of the positive Wassermann test; the sugar content is well within the normal range. The small group having purulent meningitis (Table V) shows absence of sugar or very low sugar content and is differentiated from those having tuberculous meningitis by the demonstration of the causative bacteria either by smear or culture.

TABLE VI

TUBERCULOUS MENINGITIS*

CASE	CLINICAL DIAGNOSIS	CELL COUNT	GLOBULIN, GRADE	SUGAR, MG.	SMEAR FOR BACILLI OF TUBERCULOSIS	GUINEA PIG INOCULATION	REMARKS
1	Tuberculous meningitis	165	1+	28	−	+	Necropsy
	(four days later)	98	1+	22	−	+	Necropsy
2	Undetermined	33	3+	0	−	+	
3	Poliomyelitis	248	4+	10	−	+	
4	Puerperal sepsis	450	4+	37	−	+	
	(two days later)	430	4+	28	−	+	
	(five days later)	320	4+	22	−	+	Necropsy
5	Tuberculous meningitis	500	4+	5	+	+	
6	Tuberculous meningitis	101	4+	0	−	+	
7	Undetermined	278	2+	37	+	+	
8	Meningitis	43	1+	40	−	+	
9	Tuberculous meningitis	120	2+	0	+	+	
10	Pulmonary tuberculosis	6	1+	31	−	+	Necropsy
11	Encephalitis	192	1+	18	−	+	
12	Brain abscess	180	4+	35	+	+	
13	Basal meningitis	97	3+	60	−	+	
	(five days later)	230	2+	55	−		
	(ten days later)	230	3+	43	+	+	
14	Subacute endocarditis						
	(postmortem)	18	1+	5	−	+	Necropsy
15	Influenza	400	2+	11	−	+	
		300	2+	13	−		
16	Pyloric stenosis	70	1+	60	−	+	Necropsy
	(three days later)	90	3+	48		+	
	(eleven days later)	90	2+	31	−		
17	Encephalitis	42	1+	25	+	+	Necropsy
18	Poliomyelitis	45	2+	0	−	+	
19	Meningitis	140	2+	25	−	+	
20	Tuberculous meningitis	110	3+	19	−	+	
21	Tuberculous meningitis	65	3+	26	−	+	
		190	3+	8	+	+	Necropsy

*All cultures negative.

Cases of poliomyelitis and epidemic encephalitis (Table V) are placed in one group; the sugar content shows no differential variations.

There were twenty-one proved cases in the group diagnosed tuberculous meningitis (Table VI). The sugar content ranged from 0 to 60 mg. This figure is higher than has been reported. This may be due to the fact, however, that in

Cases 13 and 16 the lumbar puncture was done five to ten days before any clinical signs of meningeal irritation became apparent, but as the disease progressed the sugar content gradually fell to a low level. It is of particular interest to note the percentage of error in the clinical diagnosis on the admission of these patients to the hospital, but in most instances the clinical findings were too indefinite to diagnose them otherwise.

SUMMARY AND CONCLUSIONS

The data collected in this study corroborate recent studies on the sugar content of the spinal fluid. The normal range is probably between 60 to 90 mg. for each 100 c.c. of fluid. The present data offer no differential points between poliomyelitis, encephalitis, brain tumor and other nonbacterial affections of the central nervous system. The sugar content, however, easily distinguishes them from meningitis. While the sugar content in cases of tuberculous meningitis may be in the same range as in case of purulent meningitis, it is generally higher and seldom zero. The distinguishing point, however, between these two important groups lies in the demonstration of the causative organism either by culture or smear and this is usually easily accomplished in the cases of purulent meningitis.

In cases of tuberculous meningitis the spinal fluid is usually clear and the cells are mostly lymphocytes while in the presence of purulent meningitis the fluid is usually cloudy and the polymorphonuclear leucocytes are the predominating cells. The cell count in cases of tuberculous meningitis is seldom higher than 500, while in cases of purulent meningitis it is seldom lower than 500. In my series it has been shown that the spinal fluid yields diagnostic data long before definite clinical signs of meningeal irritation become apparent and when these are added to the clinical history the diagnosis is definite.

The sugar concentration of the cerebrospinal fluid is of valuable diagnostic significance in the differential diagnosis of diseases involving the central nervous system.

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THREE PHASES OF WASSERMANN TECHNIC: ASSOCIATED CONSIDERATIONS, A COMPARISON OF ANTIGENS, AND A METHOD OF TITRATING AND ESTIMATING POSITIVITY OF A SERUM*

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PART I

THERE are three phases of Wassermann work which we wish to discuss briefly. First, associated considerations, secondly, antigens, and thirdly, a method of titrating positivity of a serum.

Of associated considerations there are a number of factors that are deserving of some emphasis and can well be frequently reiterated,

The care of glassware is of first importance. The glassware used for Wassermann work should be kept by itself and used for that department only. It is well to soak new material overnight in 2 per cent hydrochloric acid, but it should be most thoroughly rinsed afterward and allowed to stand overnight in water, and then rinsed again before drying for use.

In the routine cleaning of glassware, acid preparations are not desirable and no form of soap need be used if care is taken. Pipettes can be placed in a tall jar of distilled water immediately after use. The distilled water is rather better than tap water in keeping away the grayish film which tends to form in the lumen of the pipette. The Wassermann tubes should be emptied as soon as the test is completed, rinsed out, and filled with water. Then they can stand until a convenient time for cleaning. The tubes can be perfectly cleaned by using plain hot water and a stiff rod with a cotton swab on the end. The cotton can be frequently changed where there are many tubes. The inside of the tubes should be thoroughly rubbed, then rinsed and drained and put to dry in the oven. A finer wire with a bit of cotton is convenient for cleaning the inside of the pipettes. After washing, they may be finally rinsed in distilled water.

It is desirable to use plenty of pipettes and a fresh one for every serum.

The next point is in regard to complement. Uniform diet for the guinea pigs such as oats, hay, carrots, some greens, as lettuce or dandelions, if they are available, helps to make satisfactory complement. The food given the guinea pigs must be in good condition. Partially decayed greens or carrots will make them sick. The guinea pigs should have no food the day they are bled for complement. Complement taken from a guinea pig with a full stomach often is low in activity and frequently gives a cloudy end-point in titration. Several tubes will not clear quite completely, and as a result an

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inaccurate amount of complement is used in the series. All workers know that the kind and amount of complement used in the test has important bearing on results. Inasmuch as complement varies in activity and in its ability to be bound by the syphilitic reagin and antigen complex, the use of a group serum becomes a real necessity. Almost every worker with a little care can arrange to use serum from as many as three guinea pigs, a fair amount from each one.

Often serums are sent to us for the Wassermann test and the sender takes the precaution to heat them before sending, indeed heats them a half hour.

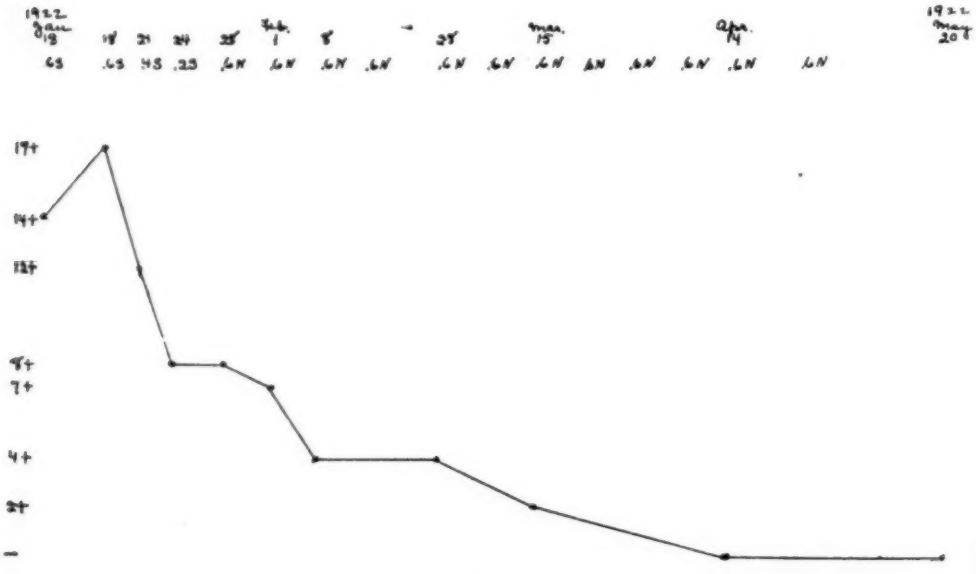

Chart 1.

Charts 1 and 2*.—Patient A., was a physician who had a sore on his thumb of five weeks' duration when he came to the laboratory. Dark-field examination of material from the sore showed the presence of the Treponema pallidum. Secondaries had not yet appeared. The Wassermann reaction was strongly positive titrating 14-plus. The patient was given German arsphenamine 0.6 gm., Jan. 13 and 18, 1922. He had severe reactions after each injection, but desired to continue and received 0.4 gm., Jan. 21, and 0.2 gm., Jan. 24. He was so ill after these injections, a change was made to neoarsphenamine and he received 0.6 gm. on Jan. 28, and Feb. 1. From Feb. 1 to April 25 he had injections of neosalvarsan at weekly or ten-day intervals. Blood for the Wassermann was withdrawn before each treatment until Feb. 8, then at varied intervals as according to Chart 1. The patient was negative April 14 and May 20. After May 20 the patient did not return for observation or treatment until July 31. The Wassermann at that time was strongly positive, titrating 13-plus as shown in Chart 2. Beginning Aug. 7, ten injections of German salvarsan were given as follows: Aug. 7, 14, 21, 0.4 gm.; Aug. 27 and Sept. 8, 0.5 gm.; Sept. 15, 0.4 gm. The patient was so ill after this injection, one week was omitted. Sept. 27 he had 0.4 gm. Again he was so ill a week was omitted. Oct. 11, he received 0.3 gm., and Oct. 18, 0.2 gm. He had such severe reactions after these injections, salvarsan was discontinued. He then began using mercurasol, which he tolerated well and he had two injections a week. On Jan. 22, 1923, the Wassermann was again negative.

The patient rested from treatment and did not return until May 31. The Wassermann was positive, titrating 12-plus. He then received mercurasol injections over a period of some weeks. He developed a sensitivity to it and ceased treatment until Oct. 13. His Wassermann was positive, titrating 6-plus at that time. Oct. 15 he had an injection of 0.75 gm. neosalvarsan and Nov. 3, 0.6 gm. He was very sick after both injections and discouraged because he tolerated treatment so poorly. His treatment since has been desultory and there has been no Wassermann test. The curves of titration are shown in Charts 1 and 2.

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*Charts 1-10.—The plus values represent the amount of positivity shown by the serum on the dates appearing at the top of the chart.

For explanation of the reckoning of positivity see Table in Part III.

The figures and letters below the dates show the drug and dosage used on those dates and between them.

S = Salvarsan.

N = Neosalvarsan.

The value and custom of the fifteen-minute inactivating period needs to appear often in print so that it may be very generally recognized as the proper procedure.

We have done a good many Wassermann tests on serums in which the

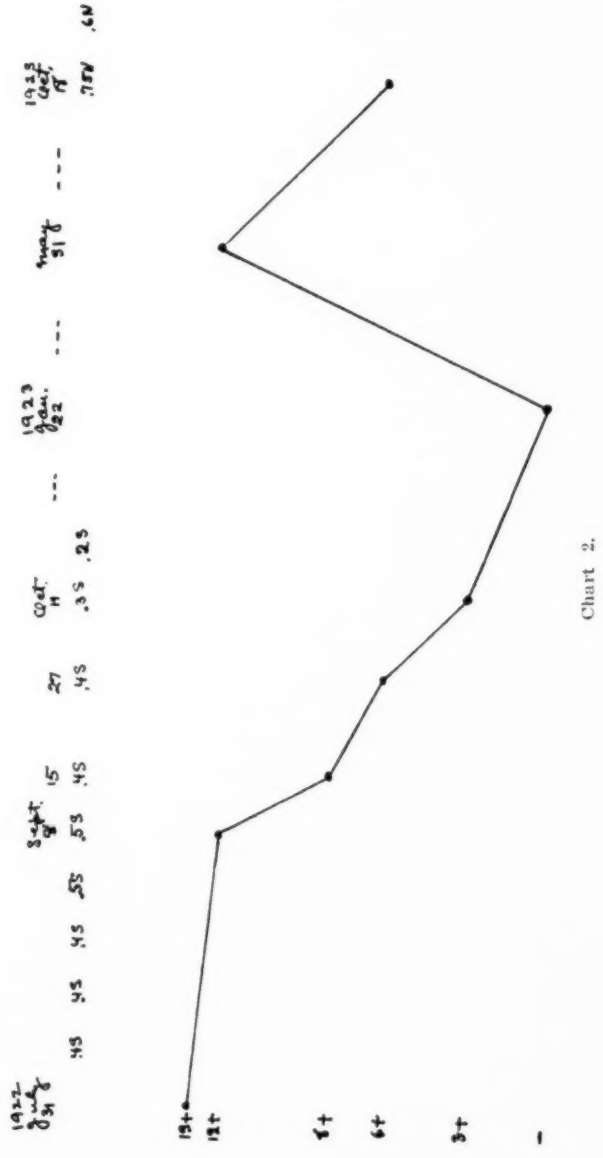

Chart 2.

native amboceptor was absorbed in one portion and not absorbed in the other. We prefer the results obtained when the native amboceptor is absorbed as a routine measure. The Kahn[1] method is very simple and effective. A drop of the washed cells is added per c.c. of serum. The tube is shaken, allowed to stand ten minutes at room temperature, then centrifuged.

The last point of associated considerations to be considered has to do with amboceptor and corpuscles.

When corpuscles are obtained from an abattoir it may happen that a supply may be obtained which is extremely difficult to hemolyze. When the complement is titrated the result is very poor, too much complement being required, and the trouble may appear to be the complement when in reality it is the corpuscles. Fresh corpuscles should be obtained.

A 2½ per cent suspension of corpuscles, just half the orginal 5 per cent suspension, is a very satisfactory percentage. We prefer the 2½ per cent to the 2 per cent recommended by Dr. Kolmer in his test because the extra ½ per cent gives just a little more color and body to the mixture. If one sensi-

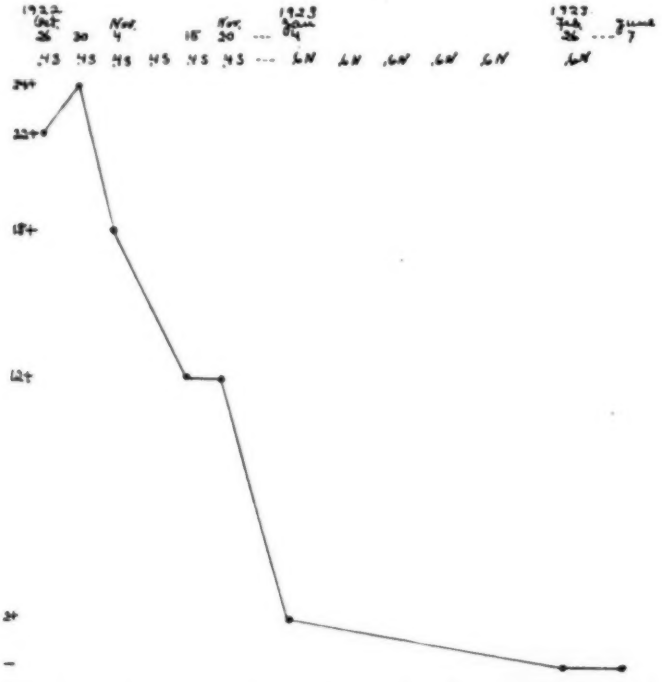

Chart 3.—Patient B. had a sore throat of several weeks' duration and the eruption of secondary syphilis when the Wassermann was done Oct. 26, 1922. The test was strongly positive with a titration of 22-plus. A series of six injections with German arsphenamine was given with 0.4 gm. dosage, beginning Oct. 26. The patient had severe reactions from the salvarsan and the treatments were discontinued between Nov. 20 and Jan. 4. Beginning Jan. 4, 1923, a course of neosalvarsan dosage 0.6 gm. was given at weekly or ten-day intervals until Feb. 26. The Wassermann was negative on that date. After the neoarsphenamine a series of about ten mercurasol injections was given. From the beginning the patient took protiodide of mercury by mouth.

The curve of titration is shown in Chart 3.

tizes the cells it is necessary to add all the corpuscles that are to be used at once to the given volume of amboceptor and mix thoroughly. For instance, if one is going to add 100 c.c. of corpuscles to 100 c.c. of amboceptor and instead of doing it all at once, pours 50 c.c. into the amboceptor, is then called to the telephone, returns and adds 50 c.c. more, the first 50 c.c. will absorb most of the amboceptor and there will not be much left for the next 50 c.c. The result will be a mixture carrying dead wood. When it is added to the

Wassermann tubes, and they come to the reading, there are apt to be false-positives and false-anticomplementary results. If the native amboceptor has not been previously absorbed in the serum, some tubes will have enough perhaps to take care of the extra cells and some may not and there may be irregular results. For an example of this, a technician once came to me who had been having trouble with his Wassermanns because he had been adding his corpuscles a few at a time to the amboceptor. If the cells are not sensitized and the amboceptor is first added and then the corpuscles, the tubes should be shaken immediately so that the corpuscles may be evenly loaded with the amboceptor and unbound complement. When the sensitized cells are prepared, a half hour at room temperature is sufficient time for the mixture to stand before use.

PART II

Because of the important place that antigens hold in the Wassermann test, we are presenting the result of some work with various antigens in two sets of five thousand cases each.

First, we wished to observe how Dr. Kolmer's antigen, as prepared in his own laboratory, compared with the usual other three antigens; the cholesterolized, the plain alcoholic and the acetone insoluble, as employed in the four-hour ice-box fixation.

Secondly, we desired to compare a group of three antigens prepared by Dr. Kolmer's[2] method, but without the use of the shaking machine, with the three types of antigens of the first set.

The Kolmer antigens of the second group were prepared after Dr. Kolmer's technic with the exception that neither the Sohxlet nor the mechanical shaker were used. One was made from Difco heart muscle powder, the other two from a mixture of three dried beef hearts for each antigen.

While our experience shows that all antigens fix to a somewhat greater degree in the eighteen-hour ice-box fixation, this work was a comparison of antigens with the four-hour fixation.

The proportions used in the test were one-half the original Wassermann quantities as follows:

Total volume 2.6 c.c.
Serum 0.1 c.c. and 0.05 c.c. portions in test and 0.1 c.c. and 0.15 c.c. in serum-control tubes.
Salt 0.5 c.c.
Antigens, complement, amboceptor and corpuscles so diluted as to use 0.5 c.c. each.
Sera were inactivated fifteen minutes at 55° C.
The native amboceptor was absorbed by adding one drop of washed sheep's cells to each 1 c.c. of serum, allowed to stand not more than ten minutes at room temperature and then centrifuged.

The antigens in the first series were 0.1 per cent cholesterinized antigen. Dr. Kolmer's antigen prepared in his laboratory, plain alcoholic antigen made similar to the Neymann-Gager[3] method, and the acetone insoluble preparation. Those used in the second series were the same type as the first with the exception that the Kolmer antigen was not prepared in his own laboratory but as previously stated. The titration of antigens was similar to the Kolmer[4] method and ten units were used in the test. Four antigen control

tubes were used with two, three, four and five times the strength employed in the test.

Complement was pooled from three guinea pigs. It was titrated by using 1 to 20 dilution, beginning with 0.08 c.c. and increasing 0.02 c.c. each tube as 0.08, 0.10, 0.12, etc., through 12 tubes. The titration was incubated one hour at 37° C. and two full units used for the test measured as follows: if the tube just cleared, in which two units of undiluted complement would be reckoned as 0.016 c.c. per tube, then 0.017 c.c. one point farther was used per tube in the series.

Ice-box fixation was four hours.

Two units of amboceptor were used according to titration. A uniform titre was employed and each new amboceptor was titrated against the previous one.

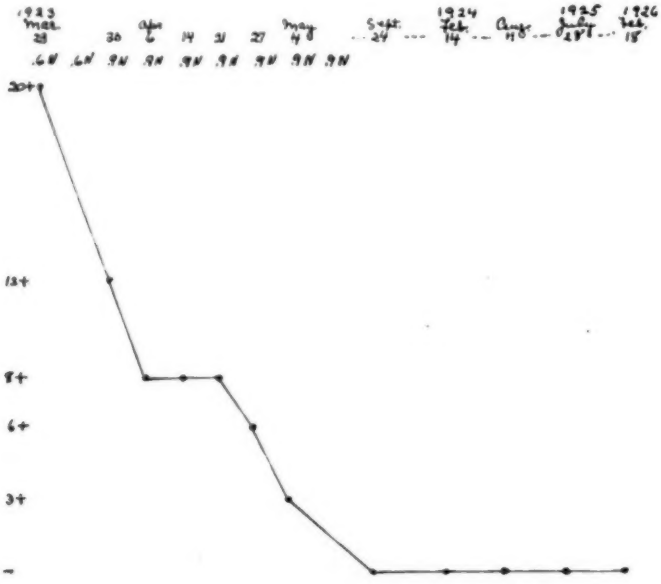

Chart 4.—Patient C. had the rash of the secondary stage of syphilis at the time the first Wassermann was made, March 23, 1923. The reaction was strongly positive, titrating 20-plus. He was given neoarsphenamine 0.6 gm. for the first two injections, then 0.9 gm. at weekly intervals until May 11. He used mercuric ointment inunctions about every other day from the first week until May. He also took the protiodide of mercury by mouth, keeping himself to the point of saturation. After May he took no more neosalvarsan and also stopped the inunctions. He continued taking mercury by mouth as much as he could tolerate until September. On Sept. 24 the Wassermann was negative. He continued the use of mercury until February, 1924. Since then he has had no treatment of any kind and his Wassermann has remained negative.

The curve of titration is shown in Chart 4.

Five per cent washed sheep's cells were used in the first series and 2½ per cent in the second series. A suitable packing of corpuscles was obtained with our centrifuge by twenty minutes on speed one. The cells were sensitized. The corpuscle suspension was strained through gauze before adding to the amboceptor.

Incubation was one-half hour in the water-bath at 37° C.

There were the usual positive and negative controls, using a moderately positive serum for the positive control.

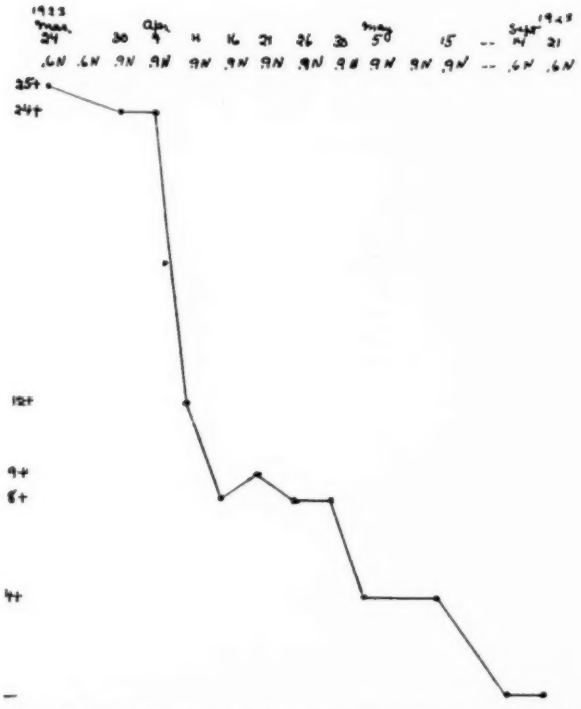

Chart 5.—Patient D. at the time of the first Wassermann was made, had a healed chancre and inguinal glands. The rash of the secondary stage had not yet appeared. The Wassermann was strongly positive, titrating 25-plus. 0.6 gm. of German neosalvarsan was given for the first treatment. 0.9 gm. was given March 27 and again March 30. From March 30 until May 15, 0.9 gm. neosalvarsan was given, usually at five-day intervals. The blood for the Wassermann was withdrawn before each treatment until May 5. After May 15, five injections of neosalvarsan were made at weekly intervals. From March to September 14 the patient had twenty-five one-grain bichloride (collapsules) injections.

At the end of August, for a period of two weeks, he took protiodide of mercury gr. ¼ four times daily and ten minims of potassium iodide three times a day. On Sept. 14 and 21 his Wassermann was negative. Sept. 14 he began a second series of neosalvarsan injections 0.6 gm. dosage. The curve of titration is seen in Chart 5.

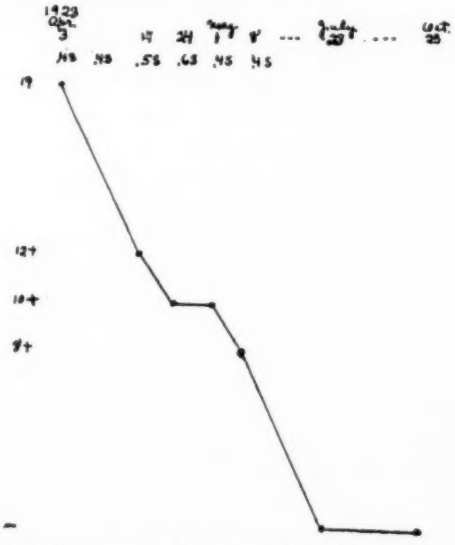

Chart 6.—Patient E. had the rash of the secondary stage of lues when he came to the laboratory for a Wassermann in April, 1923.

The Wassermann was strongly positive, titrating 19-plus. Beginning April 3, he received a course of six injections of German arsphenamine with the following dosage: April 3 and 7, 0.4 gm.; April 17, 0.5 gm.; April 24, 0.6 gm.; May 1 and 8, 0.4 gm. Following the salvarsan treatments the patient received a long course of mercury salicylate injections. The Wassermann July 23 was negative. The curve of titration is seen in Chart 6.

Those tests which gave a one-plus or a very weak two-plus with the cholesterolized antigen only, were not included in the percentage of positives. Tables I and II show the percentages.

TABLE I

	TOTAL POSITIVES	0.1% CHOLESTEROLIZED	KOLMER	PLAIN ALCOHOLIC	ACETONE INSOLUBLE
1st series	1517	1512	1471	1283	1042
5000 tests	30.34%	30.24%	29.42%	25.66%	20.84%
2nd series	1386	1385	1080	1190	826
5000 tests	27.72%	27.7%	21.6%	23.8%	16.52%

TABLE II

	0.1% CHOLESTEROLIZED	KOLMER	PLAIN ALCOHOLIC	ACETONE INSOLUBLE
1st series	1512	1471	1283	1042
1517 positives	99.67%	96.96%	84.57%	68.68%
2nd series	1385	1080	1190	826
1386 positives	99.92%	77.92%	88.85%	59.59%

The most uniform results were given by the 0.1 per cent cholesterolized antigen. Dr. Kolmer's own antigen, obtained from him, compared favorably with the 0.1 per cent cholesterolized product, but Kolmer's antigen prepared after his technic did not give as good results.

There was considerable variation between the antigenic value of Dr. Kolmer's own antigen and those made after his technic without the use of the Sohxlet or shaking machine. The omission of the mechanical shaker was the most obvious cause of variation, and differences in the value of the powdered muscle may also have been a factor. A third source of variability may have been the acetone insoluble lipoid portion of the Kolmer preparation, since there is often considerable differences in the antigenic value of the acetone insoluble antigens.

It has been brought to our attention that other workers confining themselves to the Kolmer test may be using an antigen which is not of uniform sensitivity in comparison with Dr. Kolmer's own preparation. For instance, several times we have had an opportunity to test a serum upon which some other serologist using the Kolmer technic alone returned a frank negative report. In each case, our results were four-plus positive with three antigens, including a Kolmer preparation of the second series. The positive reaction agreed with the clinical findings. In one case, a third laboratory using a group antigen system returned also a four-plus positive.

If a worker confines himself to one antigen he may be using one with less antigenic value for some time without really knowing it unless he has some means of checking his results. The titration figure is not always a sure indication of the sensitivity of an antigen.

In one of Dr. Kolmer's antigens prepared in his laboratory, ten units were represented by a dilution of 1 to 220. In another one ten units were represented by a dilution of 1 to 320. Both were sensitive. A Kolmer preparation of the second series titrated under the same conditions with 0.1 per

cent cholesterolized antigen, using the same positive sera, gave a four-plus reaction in a dilution of 1 to 2500, while the cholesterolized gave a four-plus in a dilution only as high 1-1000, yet the latter antigen was far more sensitive

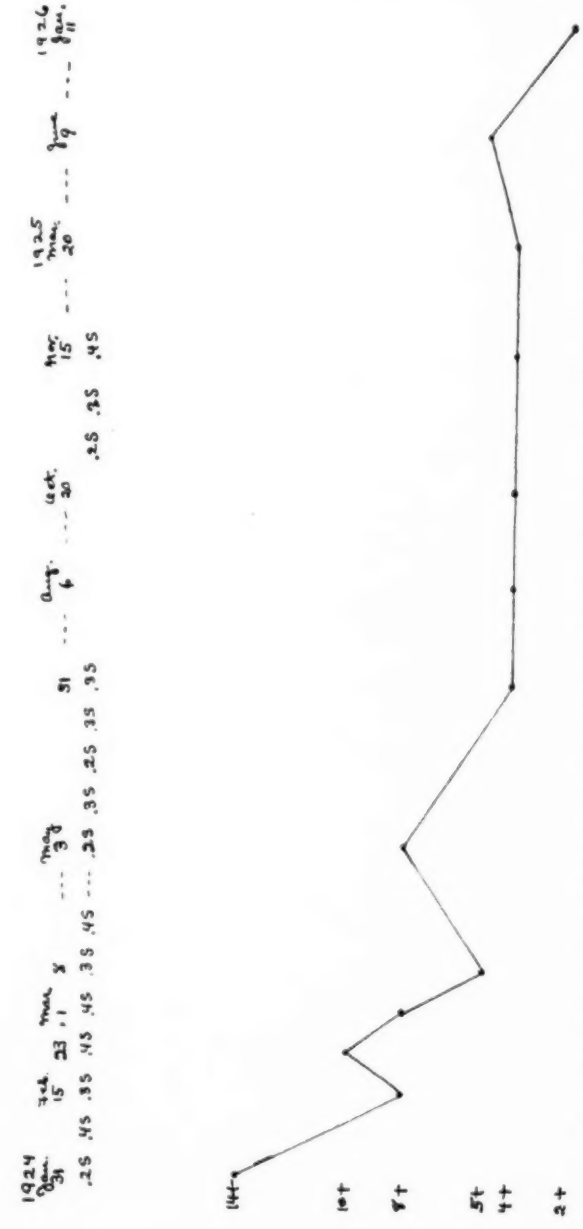

Chart 7.—Patient F., a physician, was bitten on his hand by a patient while performing a tonsillectomy in 1912. The sore was of long duration. Several weeks later he had a sore throat which healed slowly. He did not recall having a rash. On Jan. 31, 1924, he had a Wassermann done. It was strongly positive, titrating 14-plus. On Jan. 31 the patient began a course of treatment with German arsphenamine with the following dosage: Jan. 31, 0.2 gm.; Feb. 7, 0.4 gm.; Feb. 5, 0.3 gm.; Feb. 23 and March 1, 0.4 gm.; March 8, 0.3 gm.; and March 15, 0.4 gm.

The patient had no salvarsan from March 15 to May 3. Beginning May 3 he had five injections with the following dosage: May 3, 0.2 gm.; May 9, 0.3 gm.; May 16, 0.2 gm.; May 23, 0.3 gm.; and May 29, 0.3 gm. Feb. 13 and May 6 he had injections of mercurasol. Along with the salvarsan treatments and following them the patient took for many weeks, injections of oxycyanide of mercury, and much of the time the protiodide by mouth.

He began another course of arsphenamine treatments in October and received three; Oct. 27, 0.2 gm.; Nov. 5, 0.3 gm.; and Nov. 15, 0.4 gm. This course was interrupted. Since then the patient has continued the oxycyanide injections and protiodide by mouth. He has taken only short rests after mercury since he began treatment early in 1924.

The curve of titration is seen in Chart 7.

in the routine tests. The anticomplementary titration showed both antigens only slightly anticomplementary in a dilution of 1-5.

Since different kinds of antigens vary in antigenic value, and one given type of antigen may vary as prepared in different laboratories or as pre-

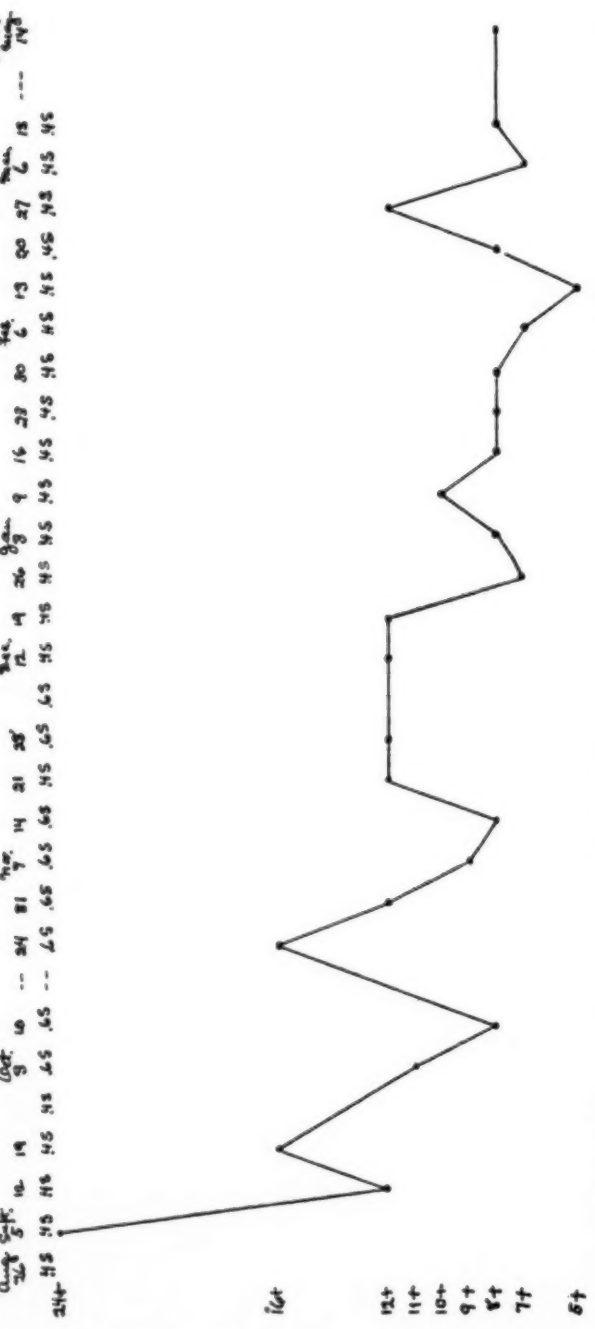

Chart 8.—Patient G. had received numerous courses of treatment but the Wassermann persisted as a strong positive. On July 1, 1922, he finished a course of twelve neoarsphenamines of 0.9 gm., dosage given at weekly intervals. He rested from medication until Aug. 26. On that day he received 0.4 gm. old German salvarsan. He had not had the old German salvarsan before and it was hoped by this change that the Wassermann would show improvement. At weekly intervals he was given 0.4 gm. until Oct. 3. The dose from then until Nov. 21 was 0.6 gm. Nov. 21 it was 0.4 gm. Nov. 28 and Dec. 5 it was 0.6 gm. From Dec. 12 to March 13, the dose remained 0.4 gm. The blood for the Wassermann was withdrawn before each treatment. The first titration was made Sept. 5, and was 24-plus. Both treatment and Wassermann were omitted Oct. 17 with a marked rise in the Wassermann curve the following week. The Wassermann was also omitted Dec. 5, but a treatment was given at that time. At the close of this long course of injections with salvarsan the Wassermann was strongly positive, titrating 8-plus. The patient was then changed to mercury medication. During the following year he took mercury by inunction or by mouth fairly continuously. He returned for a Wassermann May 14, 1924. It was strongly positive, titrating 8-plus. The curve of titration is seen in Chart 8.

pared by the same individual at different times, and since the titration figure does not always give complete information as to the sensitivity of an antigen, we incline to the view that the use of group antigens is a material aid in checking the antigenic value of an antigen and in producing more uniformly reliable Wassermann results.

PART III

There are three quantitative methods for titrating positivity of a Wassermann reaction. First, by using graduated amounts of serum and a fixed amount of complement and antigen. This is the usual procedure. Second, by using graduated amounts of antigen and a fixed quantity of serum and complement. Third, by using graduated amounts of complement and fixed portions of serum and antigen. This last method is the one we followed.

Browning and McKenzie[5, 6, 7] are particularly associated with the use of graduated portions of complement with fixed quantities of serum and antigen. In their routine tests they used three amounts of complement, 2, 4 and 6 units.

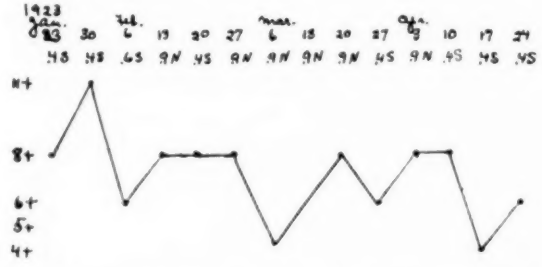

Chart 9.—Patient H., was an old case of syphilis that had received other courses of treatment. He was sent to the laboratory for a course of arsphenamine and neoarsphenamine injections. He had fourteen injections at weekly intervals. The doses were as follows: Jan. 23 and 30, 0.4 gm. German salvarsan; Feb. 6, 0.6 gm.; Feb. 13, 0.9 gm. German neosalvarsan; Feb. 20, 0.4 gm. salvarsan; Feb. 27, March 6, 13, and 20, 0.9 gm. neoarsphenamine; March 27, 0.4 gm. salvarsan; April 3, 0.9 gm. neosalvarsan; April 10, 17, and 24, 0.4 gm. salvarsan. The blood for the Wassermann was withdrawn before each injection. The curve of titration is seen in Chart 9.

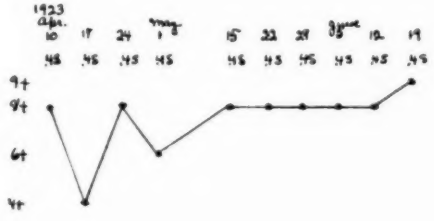

Chart 10.—Patient I. was an old case of syphilis that had received other courses of treatment. He was sent to the laboratory April 10, 1923, for a course of ten injections of salvarsan. The Wassermann was strongly positive, titrating 8-plus; 0.4 gm. German arsphenamine was given at weekly intervals with one exception. The treatment May 8 was omitted. The blood for the Wassermann was withdrawn before each injection.

The curve of titration is seen in Chart 10.

Dr. Kolmer,[8] in discussing Browning and McKenzie's method states, "it was very satisfactory and with a few modifications not involving the essential principles, yielded good results in his hands." He also states, "that the main *advantages* are the strictly quantitative character of the test and the possibility of examining anticomplementary sera; the main *disadvantages* are the expense involving the use of large amounts of complement serum where many sera are to be tested and difficulties in reading, permitting variation due to personal equation." We would mention one other objection in regard to using increas-

ing doses of complement, the fact that one is also using considerable amounts of variable biologic material.

This method was of interest to us, however, because it could be made a simple procedure and the way the complement was used, the reckoning of the degree of positivity appeared to us logical and easy.

We applied the method in the following manner:

In each tube was placed 0.1 c.c. of serum and the solution containing ten units of antigen. To each tube was added respectively, two, four, six, eight, ten and twelve units of complement, six tubes being often sufficient. Two units of complement were taken as the standard. When these tubes came to the reading, if just two units of complement were completely bound by the syphilitic reagin and antigen complex that represented a four-plus positive. If four units were completely bound that represented an additional four-plus, making a result of eight-plus. If for instance, ten units were bound the results would be recorded as twenty-plus. If only fifty per cent of tube 5 was bound the positivity was reckoned as eighteen-plus instead of twenty-plus.

Table III shows the method more graphically:

TABLE III

	TUBE 1	TUBE 2	TUBE 3	TUBE 4	TUBE 5	TUBE 6
Serum	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.
Antigen	10 units	10 units	10 units	10 units	10 units	10 units
Complement	2 units	4 units	6 units	8 units	10 units	12 units
Amount of positivity	4	8	12	16	20	24

It is necessary for the serologist to use a uniform method of titrating complement, a uniform antigen and a uniform length of fixation period for all titrations of one given case. In our series the plain alcoholic antigen was employed because we wished to use an antigen generally recognized at the time the work was begun, as giving good average results.

The general line of procedure and titration of complement in the accompanying cases was the same as outlined in Part II. The plain alcoholic antigen, as described in Part II, and four-hour ice-box fixation was employed. The complement dose was added with a 0.2 c.c. pipette graduated in one thousandths and salt was added to make 0.5 c.c. which was the complement volume per tube.

Some of the results of this work are presented in the accompanying charts.

The details of treatment are given in the cases only during the period the titration curve appeared of interest. The first six charts illustrate the titration curve in cases of early syphilis.

If the blood was procured before the first treatment and also before the second treatment, a rise in positivity was often apparent as shown in Charts 1 and 2. There was usually an abrupt descent of the curve after two or three salvarsan injections. Then there was a period of two, three, four or more treatments when the curve showed little change and this period was followed usually by a gradual descent to a negative reading.

The results in patients with late or latent syphilis, either untreated or

previously treated, were not so satisfactory. Charts 7, 8, 9 and 10 represent this type of case.

Chart 7 of patient F. was a case who had never received any previous treatment. The result indicated a general downward trend and was better than in the other three cases. They all showed a fluctuating response in the early stage of the injections and then a continued fluctuating result or a tendency to remain without much change. Titrations 8, 9 and 10 gave no indication of becoming negative. The curve in Chart 10 at the end of a course of salvarsan was higher than at the beginning.

A number of reasons may be suggested for the fluctuating curve in the old, persistently positive case. First, variability in dosage and changes from one drug to another. Second, the omission of a treatment as between October 10 and 24 in Chart 8 and between May 1 and 15 in Chart 10. In both, there was a rise in the curve. Again, the syphilitic reagin may vary in its response to the treatment, yielding temporarily, then undergo a readjustment, following somewhat the swing of a pendulum. It also may be particularly sensitive to differences in complement. Finally, the hydrogen-ion content of the salt may be a factor in variation.

When there was a marked fluctuation in the titration curve for no apparent reason, the titration was repeated to verify the result.

SUMMARY

PART I

Some routine features in connection with Wassermann technic can often be reiterated to advantage.

PART II

1. Dr. Kolmer's antigen as prepared in his laboratory compared favorably with the 0.1 per cent cholesterolized antigen with the four-hour ice-box fixation and was considerably more sensitive than the plain alcoholic and acetone insoluble antigens.

2. The three antigens prepared after the Kolmer technic without the use of the mechanical shaker showed much less antigenic value than the Kolmer antigens prepared in Dr. Kolmer's laboratory.

3. The most obvious cause of variation was the omission of the mechanical shaker. Other factors may have been differences in value of the powdered muscle and variability in the acetone insoluble lipoid portion of the preparation, since acetone insoluble antigens are known to vary markedly in sensitivity.

4. Inasmuch as antigens do vary as prepared in different laboratories and since the titration figure does not always give complete information as to the sensitivity of an antigen, the use of group antigens is an aid in checking the antigenic value of a given antigen, and in producing reliable Wassermann results.

PART III

1. The method of using fixed amounts of serum and antigen respectively and of uniformly increasing the doses of complement two units through a series of tubes offers a simple way of titrating the positivity of a serum.

2. The reckoning of positivity is made an easy procedure by adding four-plus to the positivity of a serum for every additional two units of complement which are bound by the serum and antigen complex.

3. The titration curves compiled through the treatment of a series of cases showed more uniform and satisfactory results in the case of early syphilis than in the old case either previously untreated or treated.

NOTE: We desire to express our appreciation to Dr. Josiah J. Moore, through whose courtesy and generous assistance these cases are presented.

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METASTASIS TO THYROID GLAND FROM ENDOTHELIAL MYELOMA OF BONE; RAPID REGRESSION RESULTING FROM ROENTGEN-RAY TREATMENT

BY LLOYD F. CRAVER, M.D., NEW YORK CITY*

THE case reported below has several unusual features. An interesting problem in diagnosis was presented, because the patient had an arm amputated for supposed periosteal sarcoma in one hospital, and an operation done on the thyroid in another hospital for supposed carcinoma of the thyroid, and came to Memorial Hospital with a large mediastinal tumor said to be an extension of the carcinoma of the thyroid. It appeared at once that in all probability one or both of the former diagnoses were wrong, as it was much more likely that both tumors were the same. By taking pains to secure the sections from both hospitals, it was possible to prove this assumption correct. The comparative rarity, not only of endothelial myeloma of bone, but particularly of metastases of any tumor to the thyroid gland, adds further to the unusual features of this case. Finally, the rapid regression of the mediastinal mass under roentgen-ray treatment affords an illustration of the remarkable radiosensitiveness of endothelial myeloma.

CASE REPORT

The patient was a man of thirty, a native of Holland, and was referred to Memorial Hospital on August 31, 1926, from another institution where, one month before, he had been operated on for a supposed carcinoma of the thyroid.

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Received for publication, January 11, 1927.

The history of the tumor in the thyroid was as follows: About three months before the operation he had found a small lump in the thyroid region. It had not been painful, and at times seemed to subside, until about three weeks before the operation, when the swelling had increased; and from then until the time of the operation it had persisted without remission. It had become about the size of a small orange, and involved the right lobe of the thyroid, being attached to the trachea, which was displaced markedly to the left. The mass was hard, and the patient had great difficulty in swallowing.

At operation the tumor was found to be within the thyroid substance, and was attached to the trachea and the cervical vertebrae. A mass of tumor about 8x5x4 cm. was removed, but an attempt to remove masses extending within the mediastinum was unsuccessful. The

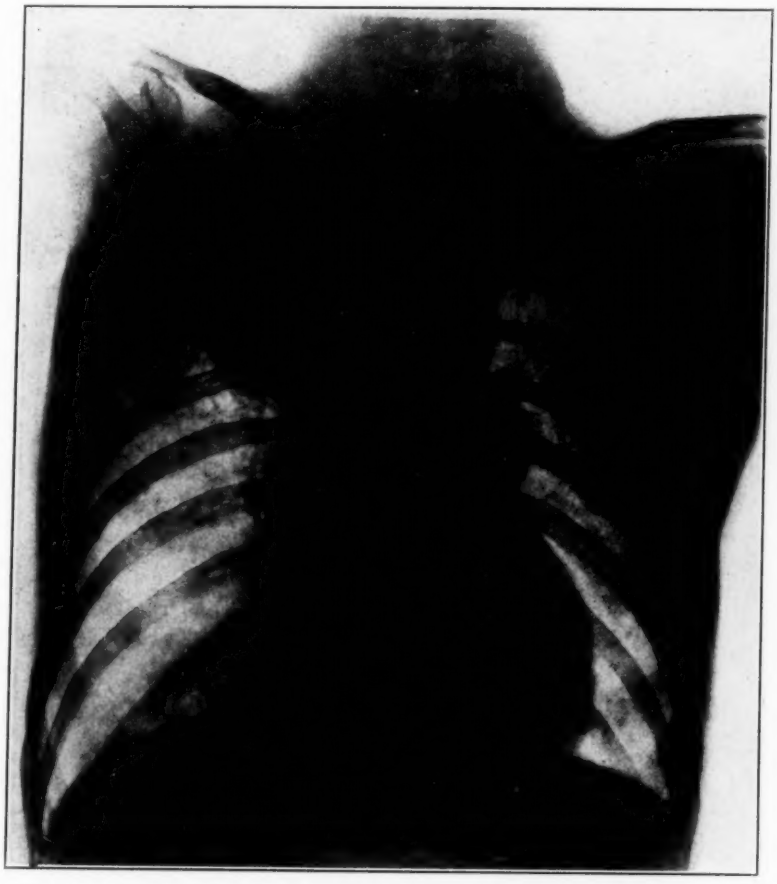

Fig. 1.—Roentgen film of chest, showing large mass in mediastinum, continuous with mass felt beneath scar in neck. Compare with Fig. 2.

section showed mostly firm dark red tissue, except at one end, where there was pale firm tissue. The microscopic examination was reported as showing carcinoma of the thyroid (probably because of the presence at one point of thyroid tissue with cystic acini containing colloid).

Upon the arrival of the patient at Memorial Hospital one month after the operation, it was evident that although he was able to be up and about, he was acutely ill. He suffered from marked dyspnea with orthopnea, and a severe cough. Under the scar of the recent operation there was still present in the thyroid region a considerable thickening; and physical signs, confirmed by the roentgen-ray (Fig. 1), indicated the presence of a large mass in the anterior mediastinum, extending further to the right, and continuous with the mass beneath the scar.

Treatment was begun immediately, without waiting to clear up the discrepancies in the diagnosis. A water-cooled high-voltage Coolidge tube was used, with the following factors: 200 kilovolts, 20 milliamperes, 50 cm. target-skin distance, and filtration of 0.5 mm. copper and 0.5 mm. aluminum. Because of the pressure symptoms it was thought unsafe to give more than five minutes, i.e., about ⅓ an erythema dose, for the first exposure. This was given directly over the thyroid and mediastinal mass anteriorly.

The remarkable effect of this treatment was strikingly shown by the prompt lessening of pressure symptoms. Because of orthopnea the first treatment had to be given to the patient as he sat in a chair. Three days later he could lie down with perfect comfort for the second treatment. A third exposure of five minutes completed the first series of treatments. The

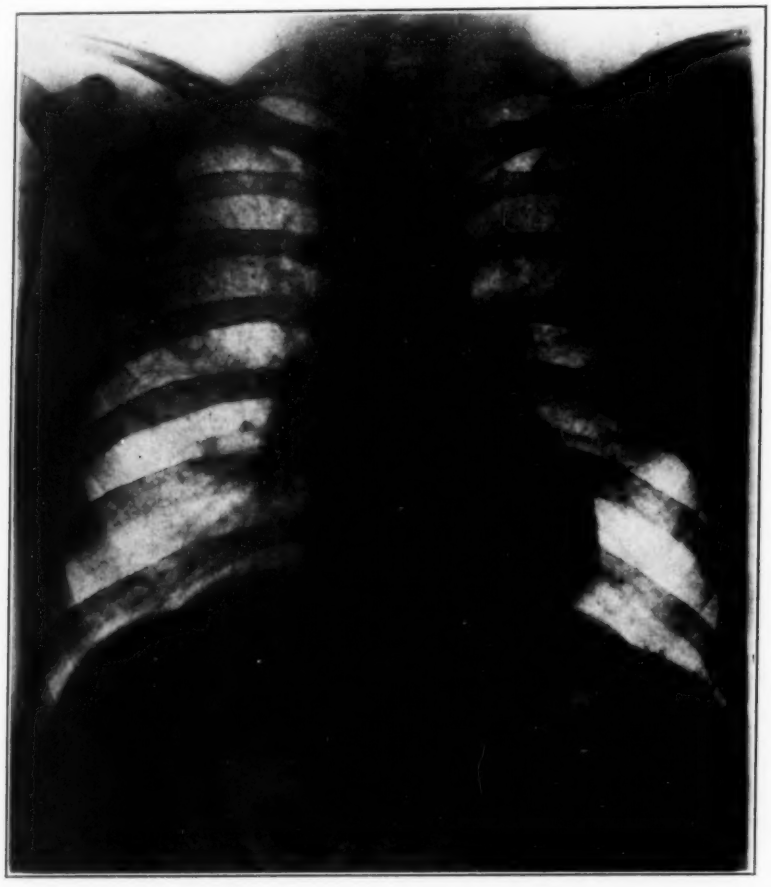

Fig. 2.—Roentgen film of chest, taken Oct. 13, 1926, about five weeks after first series of high voltage roentgen treatments. Compare with Fig. 1.

rapid regression of the tumor continued, as shown by films made ten days and about five weeks following the first series of treatments. (See Figs. 1 and 2.) Sixteen days after the third treatment an additional series of 4 exposures was given, from five to eight minutes each, to the sides of the neck and posteriorly to the mediastinum. Simultaneously with the rapid regression of the tumor there was marked relief from symptoms, in fact a complete disappearance of dyspnea and cough, and there was pronounced gain in weight and strength. The patient was soon able to resume his duties as a janitor, and has remained apparently well for over three months. Certainly even this brief palliation has been worth while.

In the meantime, further investigation of the nature of the disease was being carried out. The right arm had been amputated at a third institution 17 months before (in March,

1925). A report from that hospital revealed that nearly four years ago the right humerus was explored under a preoperative diagnosis of periostitis, the curettings having been reported as infected and necrotic pieces of bone. Again in March, 1925, the humerus had been explored, and as the pathologic report was periosteal sarcoma, the arm was amputated.

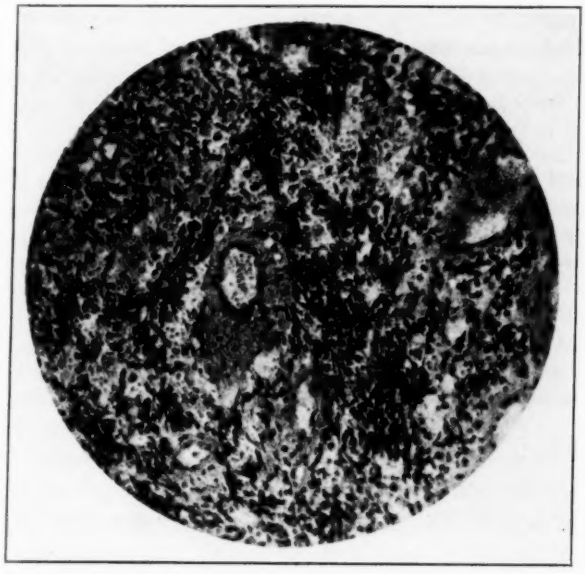

Fig. 3.—Microphotograph of section from the tumor removed from the thyroid gland. High power.

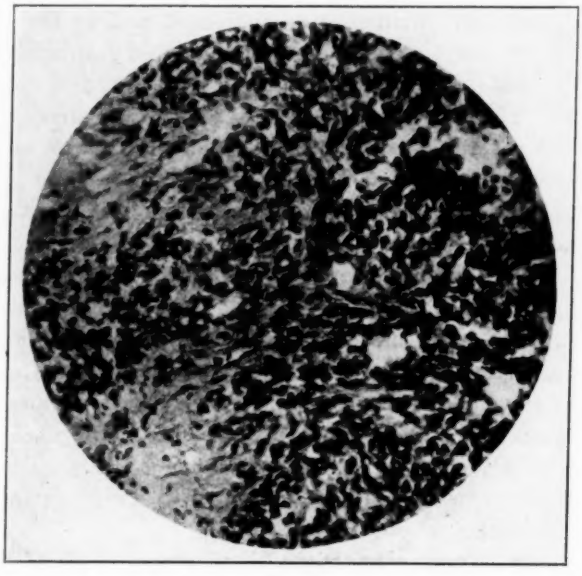

Fig. 4.—Microphotograph of section from tumor of humerus. High power.

When the patient came to Memorial Hospital, it was felt that in all probability the thyroid tumor and the tumor of the humerus were of the same origin, but it was hard to reconcile a diagnosis of periosteal sarcoma with that of carcinoma of the thyroid. Disregarding entirely the previous reports, and considering only that there had been a malignant tumor

of the humerus and of the thyroid, probably one and the same in nature, the question arose, was this a primary carcinoma of the thyroid which had first given symptoms by a metastasis to the humerus, or was it a primary sarcoma of the humerus which had metastasized to the thyroid? The absence of evidence of disease elsewhere made it seem unlikely that the third possibility need be considered, namely, a tumor arising neither in humerus nor thyroid, and metastasizing to both.

After some delay, the sections were secured from both hospitals and submitted to Dr. James Ewing, whose report follows: "The thyroid tumor is an extremely cellular, malignant anaplastic growth, identical in type with that of the humerus. It is composed of sheets of medium sized round or polyhedral cells. These cells are composed mostly of nucleus. They surround small capillaries, simulating a papillary adenocarcinoma. No definite epithelial qualities. General impression is that of endothelial myeloma." (Figs. 3 and 4.)

This case is not reported as a cure, as it is realized that the duration of the favorable result (a little over three months) is far too short to justify any such claim. Rapid recurrence is to be expected in this type of tumor, and undoubtedly further treatment will be required. The intent of this report is to put on record the combination of unusual features indicated at the beginning of this paper.

Addendum:—April 18, 1927. Further treatment to the mediastinum was given during February, 1927, because there was a slight increase in the width of the mediastinal shadow and some cough. More recently a metastasis has appeared at the base of the left lung.

SOME NOTES ON GLYCOL; GLYCOL-CHLORETONE ANESTHESIA*

By H. B. Haag and W. R. Bond, Richmond, Va.

THE last few years have witnessed an increased zeal in the quest of a suitable substitute for ethyl alcohol. Among the many substances which have been advanced for this purpose is ethylene glycol, a dihydric alcohol discovered by Wurtz in 1856. The possibilities of glycol were never strongly stressed until Bachem[1] suggested several uses, especially remarking on its adaptability as a substitute for glycerin. He noted that it could be used in the place of glycerin in preparing suppositories, ointments, and other pharmaceuticals. Administered orally to dogs in a dose of 5 c.c. per kg. body weight, no untoward effects were observed. He also stated that it had a slight laxative action, similar to that produced by glycerin. Franck[2] later advised its use as a source of food and as a substitute for ethyl alcohol. Wolff,[3] in 1920, again called attention to the possibilities of glycol in the preparation of medicinals. Curme[4] mentioned its prospects as a solvent, preservative and organic base. Fuller[5] declared that as a preservative against molds, yeasts and bacteria, it approaches ethyl alcohol, and is superior to glycerin. The substance has of late gained considerable favor as an antifreeze compound, being superior to glycerin because of its lower molecular weight.

Ethylene glycol (glycol, ethanedol) has a formula $C_2H_4(OH)_2$. Physically it is a colorless, odorless, syrupy, hygroscopic liquid having a sweet taste. It is soluble in water and ethyl alcohol, but only slightly so in ether. The compound has a boiling point of 198°C. and a freezing point of –13°C. It is somewhat more volatile and less viscid than glycerin. The specific gravity is 1.115.

*From the Department of Pharmacology, Medical College of Virginia, Richmond, Va.
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Used in our laboratory as a substitute for ethyl alcohol in the preparation of tr. digitalis, glycol, as judged by the results of several cat assays, proved somewhat disappointing. Glycol apparently does not have the same solvent action on the active principles of digitalis as does ethyl alcohol. The substitution of glycol for alcohol in spt. nitrous ether and in tr. iodine was found to be impossible. These were the only official preparations in which substitution was attempted. It was found, however, that glycol will not mix with balsam of Peru, or camphor. It will mix with menthol, ichthyol, the waters, phenol and chloretone. Aspirin is fairly soluble.

Experiments conducted in our laboratory have led us to the belief that glycol has an extremely low toxicity. Intraperitoneal administration to dogs in amounts of 5 c.c. per kg. body weight, apparently showed no injurious effects. Intravenous injections of 1.5 c.c. per kg. body weight were tolerated with little or no discomfort. Tracings of blood pressure indicate no appreciable change in pressure following the slow intravenous injection of 1.5 c.c. per kg. body weight.

For the past two years we have used a glycol solution of chloretone as an anesthetic for dogs and cats in conducting such laboratory experiments in physiology and pharmacology as the use of chloretone will permit. It has been our experience that 0.5 c.c. of a 40 per cent solution in glycol per kg. body weight injected intraperitoneally suffices to maintain the animals in a satisfactory state of anesthesia. From observations of several hundred animals, this mixture seems to possess all the advantages and none of the disadvantages of the older 10 per cent chloretone-olive oil solutions. Because of the greater concentration possible with glycol, the volume injected is decreased, making administration quicker and easier. The higher mobility of the glycol mixture allows the use of smaller needles. This and the fact that the solution is but slightly irritating makes the preliminary injection of morphine unnecessary. We have been led to believe that the glycol mixture produces its effects more quickly than the chloretone-olive oil solutions. While most of the animals injected apparently suffered no discomfort, occasionally one was encountered which reacted somewhat unfavorably, pain being marked. In these cases we believe that the peritoneum had been mechanically traumatized by the process of injection, and that this pain was not the result of irritation due to the glycol-chloretone mixture. The amount of olive oil or cottonseed oil employed in 10 per cent solutions often proved annoying in experiments on the abdominal viscera; this difficulty is obviated by use of the glycol mixture. Advantage here is due to the small amount necessary, the fact that glycol is fairly rapidly absorbed, and to its miscibility with the peritoneal fluid.

Should there be an adequate demand for glycol, it could probably be dispensed at a price lower than either glycerin or alcohol. This alone should stimulate research in an endeavor to determine its further possibilities.

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NOTES ON BASAL METABOLISM, IX.* SIMPLIFIED CALCULATION FOR GASOMETER GAS ANALYSIS METHOD†

By William H. Stoner, A.M., M.D., Philadelphia, Pa.

FORMULAS of maximum simplicity have been developed for calculating, by means of a slide rule, respiratory quotient and basal metabolic rate from the observed data of the open circuit and gas analysis method for measuring respiratory exchange.

The usual step by step calculation is the logarithmic one of Boothby and Sandiford[1] or one of its many modifications.[2-5] These methods appear to be duly laborious and time consuming. Logarithms of the various observed values and constants are added or subtracted in succession without an attempt either to collect added and subtracted logarithms or to combine constants common to every determination. In this way the respiratory quotient and basal metabolic rate are derived by a multitude of operations starting with the observed data and progressing logically to the desired result. Such a procedure, of course, makes the rationale of the calculation perfectly obvious and has been stated[6] to have the advantage of giving a complete record in each determination of all the various partial values, as ventilation per minute, calories per hour or day and calories per hour per square meter body surface. Since it is not apparent what use could be made of these partial values and since, if they were wanted, they could be obtained readily by a single slide rule setting upon the final result, it seems that this slight advantage scarcely justifies the added labor of calculating and recording. It would appear to be more expeditious to derive simple formulas based upon the rationale of the calculation and to substitute simply the observed values of a determination in these formulas for respiratory quotient and basal metabolic rate.

Several items are important in deriving simple formulas for such calculations. First, all values common to every determination should be combined into one constant.

Second, as many of the factors as possible should be made constant and

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I. Modified Clinical Method of Determination, Boston Med. and Surg. Jour., 1923, clxxxix, 193.
II. A Simplified Data Card for Clinical Determination, Boston Med. and Surg. Jour., 1923, clxxxix, 195.
III. Errors of Clinical Determination, Boston Med. and Surg. Jour., 1923, clxxxix, 232.
IV. Selection of Normal Standards, Boston Med. and Surg. Jour., 1923, clxxxix, 236.
V. Tables of Values for Dreyer's Formulas, Boston Med. and Surg. Jour., 1923, clxxxix, 239.
VI. Complementary Tables of Values of Dreyer's Formulas, Boston Med. and Surg. Jour., 1924, cxci, 1026.
VII. Actual Versus Theoretic Weight in Dreyer's Formulas, Boston Med. and Surg. Jour., 1924, cxci, 1030.
VIII. Tables of Values of the Dubois Surface Area Formula, Jour. Lab. and Clin. Med., 1926, xi, 355.

included in the one constant of the final formula. Thus, time (duration of the test), which, in the usual logarithmic forms for this calculation, is made a variable, may be made a constant, as ten, twelve or fifteen minutes, without sacrificing anything of accuracy or convenience in manipulation. Again, the gasometer factor may be included in the general constant, substituting simple linear measure of gasometer bell rise for volume in the final formula for basal metabolic rate. In this way these values, time and gasometer factor, become absorbed into the formula constant and two less operations are required in every determination.

Third, the necessity for referring to tables should be minimized. For example, the one-half of one per cent of accuracy gained in correcting a barometer reading for temperature scarcely justifies the expenditure of time consumed in consulting a table to determine whether 2 or 3 millimeters should be used in the correction of the observed barometric pressure. It would seem sufficiently accurate either to ignore this correction or to correct routinely by subtracting either 2 or 3 millimeters without reference to a table. That errors of many times this magnitude are inherent and inevitable in the determination of basal metabolic rate was shown in the third note of this series. Again, the use of logarithms for the solution of the two digit respiratory quotient or of the three digit basal metabolic rate seems unnecessarily meticulous when identical results are obtained in considerably less time by means of a slide rule or of one of the ordinary commercial adding machines. Furthermore, reference to tabular values of corrections of volumes for temperature and pressure is unnecessary. The values of normal temperature, 273 absolute, and normal pressure, 760 millimeters or 29.92 inches, may be included in the general constant of the final formula and the observed temperature and barometric pressure corrected for tension of aqueous vapor (and, if desired, for temperature) are carried as literal, variable values in the formula. Another unnecessary table generally used in the calculation of respiratory quotient is one giving volume of oxygen of inspired air corresponding to the nitrogen per cent in expired air. This table is ignored in the calculation given here. The only fact required is that the volumes of inspired and expired air are inversely proportional to their nitrogen percentages.

Fourth, the form of the final formula should be such that a minimum of kinds of operations necessary for its solution is required. For solution by means of the slide rule the simplest formula is one which has no processes of addition or subtraction which cannot be performed by inspection, and requires only multiplication and division. This statement also applies to solution of formulas by logarithms. The formulas given here may be solved logarithmically if desired.

Fifth, it is well known that, for the solution by slide rule of a formula having several simple numerical factors in the numerator and in the denominator, several settings of the rule are eliminated by alternate multiplication and division. For this reason it is advantageous to have approximately the same number of factors in the numerator as in the denominator. It is for this reason that the general constant of the final formula for basal metabolic rate given here is included in the denominator rather than in the numerator,

DEVELOPMENT OF FORMULAS

For purposes of explanation and brevity the following letters are assigned the various values specified:

C = percentage by volume of CO_2 in expired air.
O = " " " " O_2 " " "
N = " " " " N_2 " " "
v = liters of air at observed temperature and pressure expired in 10 minutes.
t = observed absolute temperature of expired air at time of measurement.
p = observed barometric pressure minus tension of aqueous vapor at t. (The scrupulous correction for barometric temperature may also be made).
V_I = liters, at normal temperature and pressure, of air inspired in 10 minutes.
V_E = liters, at normal temperature and pressure of air expired in 10 minutes.
c = calorific value of 1 liter of O_2 at normal temperature and pressure and observed respiratory quotient. (An error of approximately 1 per cent may be avoided by correcting this value on the basis of the assumption that approximately 15 per cent of the calories are derived from protein.[7-9])
f = 0.2648 N − O.
H = expected calories per day according to formulas of Harris and Benedict[1, 3, 4, 10-13] or Dreyer.[3, 4, 14-15]
A = expected calories per hour per square meter according to table of Aub and DuBois.[1, 3, 4, 11-13, 16, 17]
S = surface area in square meters according to formula of DuBois and DuBois.[1, 3, 4, 11-13, 18, 19]

A. RESPIRATORY QUOTIENT

There is a rather widespread lack of recognition of the fundamental fact that respiratory quotient is a ratio and is entirely unrelated either to the volume of expired or inspired air or to the time duration of the test. So that to determine respiratory quotient these two measurements are unnecessary so long as the duration of the test is long enough to yield a volume of expired air sufficiently large to make the sample representative. The recognition of this fact is of particular value in the more recent studies[20] of respiratory quotient curves before and after dextrose administration in the differential diagnosis between renal glycosuria and mild diabetes mellitus. In these studies, if the heat production is not required, volume of expired air and duration of test need not be measured.

Definition.—Respiratory quotient is the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed in a given time. Outside air which is inhaled in the test has the universal composition of 20.93 per cent by volume of oxygen, 0.04 per cent of carbon dioxide and 79.03 per cent of nitrogen. The volume (not the per cent) of nitrogen inhaled is equal to the volume of nitrogen exhaled.

$$\text{Volume of carbon dioxide produced} = 0.01\ CV_E - 0.0004\ V_I$$

$$\text{and " " " oxygen consumed} = 0.2093\ V_I - 0.01\ OV_E$$

$$\text{By definition, respiratory quotient} = \frac{0.01\ CV_E - 0.0004\ V_I}{0.2093\ V_I - 0.01\ OV_E}$$

$$\text{or R. Q.} = \frac{OV_E - 0.04\ V_I}{20.93\ V_I - OV_E} \quad (1)$$

Since the volume of nitrogen in inspired air is equal to volume of nitrogen in expired air,

$$0.7903\ V_I = 0.01\ NV_E \qquad \text{or } V_I = \frac{NV_E}{79.03}$$

Substituting this value of V_I in (1),

$$\text{R. Q.} = \frac{CV_E - \frac{0.04\ NV_E}{79.03}}{\frac{20.93\ NV_E}{79.03} - OV_E} = \frac{79.03\ C - 0.04\ N}{20.93\ N - 79.03\ O}$$

$$\text{or R. Q.} = \frac{C - 0.0005\ N}{0.2648\ N - O}$$

Since the value 0.0005 N is always 0.04,

$$\text{R. Q.} = \frac{C - 0.04}{0.2648\ N - O}$$

The simplest and quickest method of solving this formula has proved to be by means of a commercial calculating machine which is also used in calculating the gas analytic results. The burette readings of (1) sample, (2) volume after absorption of CO_2 and (3) after absorption of O_2 are recorded on paper and corrected. Their subtraction and the division of the remainders by the volume of the sample are performed on the calculating machine, and, immediately, from the resulting values for *C*, *O* and *N*, the respiratory quotient formula is calculated by the same machine.

B. BASAL METABOLIC RATE

Liters of oxygen at normal temperature and pressure consumed in 10 minutes $= 0.2093\ V_I - 0.01\ OV_E$.

Substituting value of $V_I = \frac{NV_E}{79.03}$.

$$\frac{0.2093\ NV_E}{79.03} - 0.01\ OV_E = 0.01\ V_E\ (0.2648\ N - O)$$

Since $V_E = \frac{v\ p\ 273}{760\ t}$ (correction for temperature and pressure),

Liters of oxygen consumed in 10 minutes reduced to normal temperature and pressure $= \frac{0.01\ v\ p\ 273\ (0.2648\ N - O)}{760\ t}$

Multiplying this value by 144 gives the oxygen consumption per day; multiplying by *c* gives the calories per day; multiplying by 100, dividing by *H* and subtracting 100 from the whole value gives the percentile variation of actual number of daily calories from the expected number which is basal metabolic rate and representing the factor (0.2648 N – O), which is the denominator in the R. Q. formula, by *f*,

$$\text{B. M. R.} = \frac{0.01\ v\ p\ 273\ (0.2648\ N - O)\ 144\ c\ 100}{760\ t\ H} - 100$$

Or,

$$\text{B. M. R.} = \frac{v\ p\ f\ c}{0.0193\ t\ H} - 100$$

The above formula applies to the Harris and Benedict standards. If Dreyer standards are used, *D* is simply substituted for *H*. If the Aub and DuBois standards are used the formula becomes

$$\text{B. M. R.} = \frac{v\ p\ f\ c}{0.463\ t\ A\ S} - 100$$

These formulas are probably solved with greatest facility by means of a slide rule but if several are to be calculated at the same time much speed may be made by use of the calculating machine to add and subtract the four place

logarithms of the values as found without writing them. As is usual in such routine calculations the characteristics of the logarithms and the decimal point in the slide rule calculation may be ignored.

SUMMARY

Simplified formulas are developed for calculating respiratory quotient and basal metabolic rate from gas analytic and gasometric data.

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LABORATORY METHODS

A STANDARD METHOD OF RECORDING THE HEMOGLOBIN CONTENT OF BLOOD*

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IN MUCH of the experimental work that is being done in various laboratories on the factors effecting changes in the blood stream many hemoglobin determinations are made. These determinations are made on the blood of different species of animals and several different hemoglobinometers are made use of. It has occurred to us that data presented from various sources would be more uniform and carry more real information if a standard method of recording hemoglobin values were followed.

The earlier hemoglobinometers were elaborated chiefly for use in clinical laboratories where examinations on human blood were made. It is understandable, therefore, that the custom arose of expressing results in terms of a so-called "normal" for human blood. Many different hemoglobinometers, however, have now come into use and with them almost as many standards. These standards vary depending upon the amount of hemoglobin chosen to represent the normal amount in human blood. Thus the reading of 100 per cent from two different instruments does not represent the same amount of hemoglobin unless they happen to make use of the same standard for human blood, which is unusual. For example, 100 per cent of hemoglobin would equal the following grams of hemoglobin per 100 c.c. of blood in the different instruments.[1]

Dare	13.77
Haldane	13.80
Oliver	15.00
Von Fleischl-Miescher	15.80
Tallqvist	15.80
Sahli	17.20
Newcomer	16.92

The confusion is further increased when one attempts to express the amount of hemoglobin found in the blood of different species in terms of percentage of a normal for human blood. Thus the hemoglobin contained in the blood

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of a normal rabbit or a normal chicken might read well below 100 per cent of the standard while that of a normal rat might read well over 100 per cent. This situation has been brought home to us since we have had occasion to make numerous determinations on different species in this laboratory.

From a chemical standpoint it would seem to be more logical to express the amount of hemoglobin found as grams per 100 c.c. of blood. This means of expression is that commonly used for many other blood constituents and has been proposed previously as a method that should be followed in recording hemoglobin values. Williamson[2] as early as 1916 made the suggestion and recently many clinicians[3] have made a strong plea for this standard means of expressing the amount of hemoglobin.

The Von Fleischl-Miescher hemoglobinometer gives a reading of grams per 100 c.c. directly and the Newcomer instrument gives a similar reading when a special conversion table is used; but in many of the other instruments only the reading of the per cent of normal is given. When using these instruments the percentage must be multiplied by the standard for the particular instrument in order to obtain grams per 100 c.c.

When all investigators report their figures in grams per 100 c.c. of blood the results from all laboratories may be compared very easily without laboriously determining what instrument was used and upon what standard that instrument is calibrated. There is one precaution, however, that should be taken. All instruments should be standardized against Wong's[4] iron method or Van Slyke's[5] oxygen capacity method of determining hemoglobin. If all instruments are not properly calibrated figures will be of no more value than when the per cent of normal was used.

We have not space here to discuss the accuracy of the various methods of determining hemoglobin and it is unnecessary for both Robscheit[6] and Senty[7] have made excellent criticisms of the common methods in use, but we do wish to mention our experience in this laboratory. We have standardized our Von Fleischl-Meischer and Newcomer instrument with Wong's iron method and find them to check well within experimental error, but in standardizing our Dare instrument we encounter the same difficulties reported by Lindsay, Rice, and Sellinger. We have therefore discarded the Dare in all our work except with our chicks. With them all readings are below 65 per cent and at such a low reading fairly accurate results are obtained. In this case, however, we make a definite note of the fact that the Dare instrument was used and hesitate to compare the figures with those obtained by the use of the other instruments.

SUMMARY

We suggest that all hemoglobin determinations be reported in grams per 100 c.c. of blood.

Reasons for the necessity of making this change and the value derived from using this standard method are given.

The need for proper standardization of instruments, and the precautions which should be taken in dealing with figures from an improperly calibrated instrument are pointed out.

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FLOCCULATION TESTS IN THE SEROLOGY OF SYPHILIS

By Dr. Ernst Meinicke*

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UP TO 1917 all attempts have failed to improve or replace the Wassermann reaction with a flocculation reaction. None of the many recommended tests have been satisfactory in practice.[1] The causes of the failure were the unknown experimental elements for such reactions.

For the first time in 1917, I published a test for syphilis[2] and investigated systematically the experimental elements of the lues-flocculation tests on the whole. I determined the properties of the organ extracts, and the sera, and examined the influence of temperature of different salt solutions, the inactivation of the sera, the dosage of the reagents, etc.[3] I succeeded in offering three different methods of lues diagnostics. The first method (Wassermethode) favors flocculation in negative sera only; the second method (Kochsalzmethode) favors flocculation in all sera, the floccules dissolving upon addition of salt (sodium chloride) in the negative sera, and staying in the positive sera. As third method I chose an arrangement which caused flocculation in the positive sera only.

It may be mentioned that I developed the "Kochsalzmethode" to an immunity reaction in cooperation with Bley[6] and Neumann.[7] I used the method in the diagnosis of malleus (Glanders) of the horses with best result. To the alcoholic organ extract we added an antigen from B. mallei, and mixed this with sera from infected and healthy horses. Flocculation was formed in the sera of the horses infected by malleus which stayed upon addition of sodium chloride; but the floccules in the sera of healthy horses dissolved upon addition of sodium chloride. The complex malleus antigen—malleus antibody—is proved by the fact that in analogy to the positive lues reaction there are formed floccules resistant to sodium chloride, probably due to a compound of the extract lipoids and serum bodies. I called the new immunity reaction "lipoid-binding reaction." Dahmen[8] used the method with best result for the diagnosis of trypanosomiasis equiperdum and the specific interstitial pneumonia of cattle. Denker[9] used it for the diagnosis of the contagious stool of cattle. The "lipoid-binding reaction" is equivalent to the agglutination or the complement-deviation as specific of all contagious diseases, and as specific differentiation of albumin. It is an immunity reaction sui generis.

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After Sachs and Georgi[10] convinced themselves of the correctness of the experimental elements of the flocculation reactions, which I elaborated and demonstrated, and published in 1918, this method has spread widely ever since under the name "Sachs-Georgi Reaction" (S.G.R.) The S.G.R. asks for cholesterinized extracts from cattle heart which Sachs used for the Wassermann reaction. The test is done in the medium of a physiologic salt solution. In 1919 I recommended for practical use the "third modification"[11] of my flocculation reaction which gives flocculation in the positive sera only. This method is usually called "D.M." in numerous laboratories. It is characteristic for the "D.M." that the extracts are obtained from horse heart which are extracted first by ether and then by alcohol. The reaction is done in the medium of a hypertonic salt solution.

Concerning the preparation of my extracts and the technic of the "D.M." I refer to my previous publications.

Following the example of the "D.M." and "S.G.R." all later lues reactions have been elaborated and recommended by a number of authors. The methods differ only in the choice of the organ extracts, in the variety of the additional agents, in the different salt content of the media, and the different ratio between the volume of serum and extract used. As far as the preparation of the extracts is concerned the previous extraction with ether or similar chemicals I recommended, has proved satisfactory. The addition of cholesterin, as recommended by Sachs, is frequently used to strengthen the extract; or both have been combined. Among the numerous authors, reactions have been recommended by Hecht, Bruck, Kodama, Vernes, Dreyer, Ward, and Kahn. An improvement in the reactions has been attempted by reducing the amount of extract to a smaller volume in comparison to the amount of serum used; this pertains to the reactions recommended by Hohn[14] and Kahn.[15]

In the meantime it has been made a particular study to convert the lues reaction into speed reactions (Dodd[16]). As is known a Wassermann reaction is completed within a few hours while the flocculation methods always asked for an entire day. This was a disadvantage, therefore I elaborated a speed reaction, the "Meinicke-Trubungsreaction" (M.T.R.)[17, 18, 19] which is characterized by the addition of balsam of Tolu to the organ extract. I chose balsam of Tolu because it appeared to me very suitable to improve the accuracy and speed of the reaction. Many improvements and changes in the flocculation tests have been advised; it is impossible to describe them all. None of them offer anything new in principle.

Generally speaking the following comments may be made from previous experiences:

1. None of the methods described hitherto will replace the Wassermann reaction entirely. There are cases of lues in which flocculation tests fail, and the Wassermann reaction only is positive.

2. According to the scientific opinions of today, it is not justified that laboratories operate the Wassermann reaction only. There are many cases of primary and latent lues where the Wassermann reactions fail, and the flocculation and precipitation tests are positive. At least one of the substitute methods should always be operated at the same time with the Wassermann reaction.

3. The accuracy of the reactions is greatly improved by operating different methods at the same time, and the number of false readings are reduced to a minimum.

PREPARATION OF THE EXTRACT

Horse heart is finely powdered and dried; the powder is extracted first by ether then by alcohol; the degree of concentration of the ether-rest-extract is determined empirically; the extract is diluted with alcohol, 96 per cent, to about 1:14; then balsam of Tolu and benzoic acid are added. The extracts are to be kept at room temperature protected from light.

DILUTION OF THE EXTRACT

A quantity of extract sufficient for the number of tests intended is put into a tube, and ten times the quantity of a 3 per cent sodium chloride solution containing 0.01 per cent crystallized sodium carbonate in another tube. Both tubes are warmed in a water-bath for five to ten minutes to a temperature of 45°; then the solutions are mixed rapidly by pouring the salt solution into the extract; the mixture is then poured back into the empty tube to insure thorough mixing.

SERA

The sera must be made perfectly clear by centrifugalization. They are not to be inactivated, but must be used active.

TECHNIC OF THE TEST

To each 0.2 c.c. serum is added 1.0 c.c. of the freshly prepared extract dilution, after the latter has ripened for a few minutes. The test tubes stay for one hour in a warm room (20° Celsius).

READING OF THE RESULTS

The examiner stands before a light window in a distance of about 2 to 3 meters, and watches the crossbar of the window through the reagents in the test tubes. In gloomy weather the readings are done by artificial light.[19c] Similar to the x-ray plate illuminating cases, a small case with two hidden bulbs may be used. The front side of the case is closed to an opening of 15 × 20 cm., into which is fastened a plate of opaque glass covered with a broad black cross. The reagents in the test tubes are examined by watching the cross in the glass through the light, holding the test tubes at about 60 cm. distance from the case.

Reactions are negative if the transparency of the fluid is unimpaired. The cross has distinct straight lines, and appears black. In strongly positive reactions the fluid is entirely opaque; the cross cannot be seen. In weakly positive reactions the cross appears in grey color with indistinct contours, as if it were lying behind a veil.

In small laboratories where only few specimens are tested at one time, or in weakly positive cases, or if preferred for other reasons, a control may be used. Each specimen is run with two tubes, one for the test proper, the

other one for the control. The only difference is the addition of one drop of formaldehyde, 40 per cent, in the control tube at the beginning of the test. If the main tube has the same appearance as the control tube, the specimen is negative. If the main tube is more opaque than the control tube the specimen is positive, the degree of positiveness depending upon the degree of opaqueness.

The young author, Dohnal, who lately died in Innsbruck, has converted my "Trubungsreaktion" into a microreaction.[21] My assistant, Dr. Gross,[22] and I[20] have added further improvement to this new method; the "Meinicke-Miera reaction" only in a somewhat weaker serologic adjustment. The dilution of the extract is done in the same manner as described above.

SERA

Sera must be active. Minute quantities of blood, as may be drawn into a capillary from a needle prick into the finger tip or the ear lobe, are sufficient for the test.

TECHNIC OF THE TEST

Mixing the diluted extract and the serum is best done by means of gauged platinum wire ring inoculating needles. After the fresh extract-dilution has ripened for a few minutes it is poured into a small porcelain dish. By means of platinum needle with an eye of 5 mm. diameter a drop of extract dilution is taken. By means of another loop with a diameter of only 2.5 mm. a drop of serum is mixed within the lumen of the larger needle with the diluted extract. With the smaller loop a drop of the mixture is then placed upon a cover glass on a hollow slide, and left for three-fourths to one hour at a temperature of 20° to 22° C.

READING THE RESULTS

The drops are examined microscopically with a weak eyepiece and a strong dry objective (for instance: Leitz eyepiece 1 or 2, and dry objective 6 or 7).

The different layers of the drop must be focalized and examined.

The reaction is negative if one sees either nothing or numerous exceedingly minute vivid particles in molecular movement. Strong positive reactions are characterized by the appearance of thick floccules which sink to the bottom layers of the drop due to their gravity, and become the larger the closer they lie to the bottom. In weak reactions the microscopic field is covered with more or less large floccules lying close to one another which are larger on the lower pole of the drop than in the upper layers.

Very elegant pictures are seen by examining the "hanging" drop in the dark-field as recommended by Hartwich.[23] If the necessary lenses are not available, one may examine a layer preparation in the dark-field instead of the "hanging" drop. A reaction is negative if one sees an immense number of uniformly distributed light dancing particles in a grey field. A reaction is positive if one sees scattered more or less large jagged brilliant white floc-

cules in a deep black field. Between these findings there are all shades according to the intensity of the reactions.

The macroreaction (M.T.R.) and the microreaction (M.M.R.) are exceedingly convenient methods for the diagnosis of syphilis and offered to the free use of the profession. Everybody who is acquainted with the use of a pipette and the simplest serologic technic, can operate these methods. Reading the results does not require any more practical experience than the determination of albumin in the urine. The observation of the microreaction requires the knowledge of the usual microscopic technic only.

There is no need for an incubator and an exactly adjusted water-bath for the inactivation of the sera. The microreaction can be done even without a centrifuge. If the blood specimens are collected in narrow glass tubes, they may be left overnight, while the serum will separate from the coagulum. By means of the eye of a platinum wire needle a minute quantity of the clear serum is obtained. . To operate the M.M.R. one needs only a few glass containers, a few platinum inoculating needles, and a microscope.

The elegance and simple technic of my methods have been recognized in the literature. I refer to the original articles of, for instance, Klopstock.[24] "There are a large number of statements which speak in highest terms of the elegance and speed of the Meinicke-Trubungs-reactions." I further refer to the original articles of Untersteiner,[25] Prochazka,[26] Oro Augusto,[27] Poschacher,[28] Pais,[29] Kirchner,[30] Ivanoff,[31] Hager,[32] Peterson, Saunders,[33] Szirmai,[34] Laubenheimer and Hamel,[35] Van der Hoeden,[36] Schilling,[37] Tedeschi,[38] Untersteiner,[39] Rüder,[40] Strempel,[41] and Mefford.[91]

Relative to the sensitiveness of my reactions Klopstock and Hilpert[43] make the following statement in their comprehensive article, "Most original articles agree on the fact that the Meinicke Trubungs-Reactions have a high sensitiveness." For further information it may be referred to: Delitala,[44] Panofsky,[45] Beretvas,[46] Mylius,[47] Klaften,[48] Alexander and Emmich,[49] Bering,[50] Förtig,[51] Klein,[52] Kruspe,[53] de Benedetti,[54] Behrmann,[55] Schükri,[56] Fabian,[57] Richter,[58] Klopstock and Dölter,[59] Elkeles,[60] and others.

According to my own experiences my methods agree in about 95 per cent of all cases with the Wassermann reaction. Of course the percentage is dependent upon the type of syphilitic patient examined. The more recent cases of lues, controls of treatment, and latent cases of lues are to be examined, the more frequent quantitative and qualitative differences will be observed in the use of different methods. But they never exceed 10 per cent according to my experiences. As far as different reacting syphilitic patients are concerned, the M.T.R. and M.M.R. are superior to the Wassermann reaction, in about two-thirds of the cases in sensitiveness; the Wassermann reaction, is positive in only one-third of the diverging cases with negative results of my methods.

Concerning the specificity of my reactions I refer to the literature, especially to: Förtig,[61] Pomini and Cascella,[62] Schultze,[63] Kruchen,[64] Klopstock and Hilpert,[65] Boas,[66] Jaenecke,[67] Sato,[68] Schubert,[69] Untersteiner,[70] Hilgers,[71] Elkeles,[72] Mutermilch,[73] Austerweil,[74] Rocca,[75] Knebel,[76] and others. Authors like Klopstock, who in his department of Sachs' Institute reexamined the M.T.R. and who discovered a few unspecific reactions in the beginning, re-

cently admitted freely that the specificity of the M.T.R. is preserved well. He states that the M.T.R. in its recent form is a very valuable reaction for syphilis, and has great advantages over the other methods.

I especially quote Klopstock's statements, as they are based upon very large material, and originated from Sachs' Institute, where the S.G.R. and the Benzochol-reaction have been discovered, competitive methods of my reactions. The criticism of Sachs' school on my methods appears to me especially valuable.

The practical usefulness of the M.T.R. and the M.M.R. has been proved. For further information on the microreaction (M.M.R.) I refer to: Dohnal,[77] Niederwieser,[78] Spicca,[79] Loevy,[80] Martin,[81] Hilgers and Kötzing,[82] Hartwich,[83] Burtscher,[84] Post,[85] and Peterson-Saunders.[92] In 1925 the number of sera examined in the different laboratories by the M.T.R. and M.M.R. far exceeded one million.

The main advantage of my reactions, however, is their cheap and simple technic and the speed of their operation. I explained previously the disadvantages that are necessarily attached to any centralizing institute. The main disadvantage is the limitation of examinations to clinically suspected cases only, where anamnestic data have pointed to lues. Up to the present only a comparatively small number of patients have been examined for syphilis. Therefore numerous cases of syphilis were not diagnosed, and received the wrong treatment. Sources of infection were not detected, and gave occasion for further spreading of syphilis. Now every hospital, which has a doctor who is acquainted with the simple manipulations of laboratory technic, is able to examine systematically the entire material of patients for syphilis. The same opportunity is given to the private physician who is able to build up a small laboratory with small means.

I asked Köster,[86] and Köster and Amend[87] to publish the experiences that we obtained from a systematic examination for lues of all patients of a tuberculosis sanitarium. Not more than at the highest one-third to one-fourth of the syphilitic patients admitted to the sanitarium were clinically suspicious of syphilis. The other two-thirds to three-fourths were detected by systematic serologic examination only. Köhn von Jaski,[88] Hager,[89] and Eicke[90] had exactly the same experience in their tuberculosis sanitarium. The majority of their syphilitic patients were found out by systematic serologic examination only. Antiluetic treatment greatly improved the apparently serious cases of tuberculosis. All these authors emphasize the high specificity of the M.T.R., that always gave specific reactions regardless of the seriousness of the tuberculous cases examined.

Following these favorable experiences, a number of German government-boards have recently ordered the systematic examination with my reactions on all patients of their hospitals and sanitariums. We are here at the beginning of a development that certainly will be followed by great results. The blood examination for syphilis should be done with same regularity as the examination of the urine for albumin and sugar. To create the methods for the systematic examinations for syphilis was my aim.

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TABLES FOR CALCULATION OF COLOR INDEX, VOLUME INDEX AND SATURATION INDEX BASED ON RECENTLY DETERMINED STANDARDS*

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REDETERMINATIONS of the hemoglobin, red cell count, cell volume, color index, volume index and saturation index standards have shown that the figures generally given in the texts are incorrect, and a study of the literature reveals that they have never had a very sound experimental basis. Obviously, the newer figures, based on the examination of the bloods of 137 healthy young men[1] and 100 healthy young women[2] by the most accurate methods available, should be used in all index calculations until study of a larger series of cases can give us still more accurate figures. While these standards are based on examinations of young adults, experience has shown that no great error is involved in using them for older individuals until further work establishes definite standards for these ages.

Table II is so designed that the results of blood studies can be converted into terms of per cent of the new standards without calculation, and Chart 1 is so constructed that the various indexes may be found from these data by inspection without calculation.

DISCUSSION OF THE STANDARDS ON WHICH TABLE II IS BASED

Accurate red cell counts, hemoglobin estimations, and cell volume determinations on the oxalated venous blood of 137 healthy young men and 100 healthy young women gave the results in Table I.

Table I

	RED CELLS MILLION PER C.MM.		HEMOGLOBIN GM. PER. 100 C.C.		CELL-VOLUME C.C. PACKED CELLS PER 100 C.C. OF BLOOD	
	Average	Results in 90% of the cases	Average	Results in 90% of the cases	Average	Results in 90% of the cases
Men	5.4	4.7-6.1	15.8	14.0-18.0	45	40-50
Women	4.8	4.3-5.3	13.7	12.0-15.5	41	37-45

We retain the use of five million as 100 per cent red cells in the index determinations simply for convenience in calculation. Any other figure would do, if it were generally agreed upon. Of course, five million is not the average red cell count for either normal men or women. If five million is taken as 100 per cent red cells, then the average *hemoglobin coefficient* (a term introduced by us for the number of grams of hemoglobin per 100 c.c. of blood calculated to a red cell count of five million) must be taken as 100 per cent

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TABLE II

1.	2.	3. MEN 4.		5. WOMEN 6.		7.
PER CENT	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN, GRAMS PER 100 C.C.	VOLUME OF PACKED CELLS, C.C. PER 100 C.C.	HEMOGLOBIN, GRAMS PER 100 C.C.	VOLUME OF PACKED CELLS, C.C. PER 100 C.C.	HEMOGLOBIN, GRAMS PER 100 C.C.
10	0.50	1.47	4.10	1.43	4.30	1.38
11	0.55	1.62	4.51	1.57	4.73	1.52
12	0.60	1.76	4.92	1.71	5.16	1.66
13	0.65	1.91	5.33	1.86	5.59	1.79
14	0.70	2.06	5.74	2.00	6.02	1.93
15	0.75	2.21	6.15	2.15	6.45	2.07
16	0.80	2.35	6.56	2.29	6.88	2.21
17	0.85	2.50	6.97	2.43	7.31	2.35
18	0.90	2.65	7.38	2.57	7.74	2.48
19	0.95	2.79	7.79	2.72	8.17	2.62
20	1.00	2.94	8.20	2.86	8.60	2.76
21	1.05	3.09	8.61	3.00	9.03	2.90
22	1.10	3.23	9.02	3.15	9.46	3.04
23	1.15	3.38	9.43	3.29	9.89	3.17
24	1.20	3.53	9.84	3.43	10.32	3.31
25	1.25	3.67	10.25	3.58	10.75	3.45
26	1.30	3.82	10.66	3.72	11.18	3.59
27	1.35	3.97	11.07	3.86	11.61	3.73
28	1.40	4.12	11.48	4.00	12.04	3.86
29	1.45	4.26	11.89	4.15	12.47	4.00
30	1.50	4.41	12.30	4.29	12.90	4.14
31	1.55	4.56	12.71	4.43	13.33	4.28
32	1.60	4.70	13.12	4.58	13.76	4.42
33	1.65	4.85	13.53	4.72	14.19	4.55
34	1.70	5.00	13.94	4.86	14.62	4.69
35	1.75	5.15	14.35	5.00	15.05	4.83
36	1.80	5.29	14.76	5.15	15.48	4.97
37	1.85	5.44	15.17	5.29	15.91	5.11
38	1.90	5.59	15.58	5.43	16.34	5.24
39	1.95	5.73	15.99	5.58	16.77	5.38
40	2.00	5.88	16.40	5.72	17.20	5.52
41	2.05	6.03	16.81	5.86	17.63	5.66
42	2.10	6.17	17.22	6.01	18.06	5.80
43	2.15	6.32	17.63	6.15	18.49	5.93
44	2.20	6.47	18.04	6.29	18.92	6.07
45	2.25	6.62	18.45	6.44	19.35	6.21
46	2.30	6.76	18.86	6.58	19.78	6.35
47	2.35	6.91	19.27	6.72	20.21	6.49
48	2.40	7.06	19.68	6.86	20.64	6.62
49	2.45	7.20	20.09	7.01	21.07	6.76
50	2.50	7.35	20.50	7.15	21.50	6.90
51	2.55	7.50	20.91	7.29	21.93	7.04
52	2.60	7.64	21.32	7.44	22.36	7.18
53	2.65	7.79	21.73	7.58	22.79	7.31
54	2.70	7.94	22.14	7.72	23.22	7.45
55	2.75	8.08	22.55	7.87	23.65	7.59
56	2.80	8.23	22.96	8.01	24.08	7.73
57	2.85	8.38	23.37	8.15	24.51	7.87
58	2.90	8.53	23.78	8.29	24.94	8.00
59	2.95	8.67	24.19	8.44	25.37	8.14
60	3.00	8.82	24.60	8.58	25.80	8.28
61	3.05	8.97	25.01	8.72	26.23	8.42
62	3.10	9.11	25.42	8.87	26.66	8.56
63	3.15	9.26	25.83	9.01	27.09	8.69
64	3.20	9.41	26.24	9.15	27.52	8.83
65	3.25	9.55	26.65	9.30	27.95	8.97
66	3.30	9.70	27.06	9.44	28.38	9.11
67	3.35	9.85	27.47	9.58	28.81	9.25
68	3.40	10.00	27.88	9.72	29.24	9.38
69	3.45	10.14	28.29	9.87	29.67	9.52

TABLE II—CONT'D

1.	2.	3. MEN	4.	5. WOMEN	6.	7.
PER CENT	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN, GRAMS PER 100 C.C.	VOLUME OF PACKED CELLS, C.C. PER 100 C.C.	HEMOGLOBIN, GRAMS PER 100 C.C.	VOLUME OF PACKED CELLS, C.C. PER 100 C.C.	HEMOGLOBIN, GRAMS PER 100 C.C.
70	3.50	10.29	28.70	10.01	30.10	9.66
71	3.55	10.44	29.11	10.15	30.53	9.80
72	3.60	10.58	29.52	10.30	30.96	9.94
73	3.65	10.73	29.93	10.44	31.39	10.07
74	3.70	10.88	30.34	10.58	31.82	10.21
75	3.75	11.03	30.75	10.73	32.25	10.35
76	3.80	11.17	31.16	10.87	32.68	10.49
77	3.85	11.32	31.57	11.01†	33.11	10.63
78	3.90	11.47	31.98	11.15	33.54	10.76
79	3.95	11.61	32.39	11.30	33.97	10.90
80	4.00	11.76	32.80	11.44	34.40	11.04
81	4.05†	11.91	33.21	11.58	34.83	11.18
82	4.10	12.05	33.62	11.73	35.26†	11.32
83	4.15	12.20	34.03	11.87	35.69	11.45
84	4.20	12.35	34.44	12.01	36.12	11.59
85	4.25	12.49	34.85	12.16	36.55	11.73
86	4.30	12.64	35.26	12.30	36.98	11.87
87	4.35	12.79	35.67	12.44	37.41	12.01
88	4.40*	12.94	36.08*	12.58	37.84	12.14
89	4.45	13.08	36.49	12.73	38.27	12.28
90	4.50	13.23	36.90	12.87	38.70	12.42
91	4.55	13.34	37.31	13.01	39.13	12.56
92	4.60	13.52*	37.72	13.16	39.56	12.70
93	4.65	13.67	38.13	13.30	39.99	12.83
94	4.70	13.82	38.54	13.44	40.42	12.97
95	4.75	13.96	38.95	13.59	40.85	13.11
96	4.80††	14.11	39.36	13.73††	41.28††	13.25
97	4.85	14.26	39.77	13.87	41.71	13.39
98	4.90	14.41	40.18	14.01	42.14	13.52
99	4.95	14.55	40.59	14.16	42.57	13.66
100	5.00	14.70	41.00	14.30	43.00	13.80
101	5.05	14.85	41.41	14.44	43.43	13.94
102	5.10	14.99	41.82	14.59	43.86	14.08
103	5.15	15.14	42.23	14.73	44.29	14.21
104	5.20	15.29	42.64	14.87	44.72	14.35
105	5.25	15.44	43.05	15.01	45.15	14.49
106	5.30	15.58	43.46	15.16	45.58	14.63
107	5.35	15.73	43.87	15.30	46.01†	14.77
108	5.40**	15.88**	44.28	15.44	46.44	14.90
109	5.45	16.02	44.69**	15.59	46.87	15.04
110	5.50	16.17	45.10	15.73	47.30	15.18
111	5.55†	16.32	45.51	15.87	47.73	15.32
112	5.60	16.46	45.92	16.02	48.16	15.46
113	5.65	16.61	46.33	16.16	48.59	15.59
114	5.70	16.76	46.74	16.30	49.02	15.73
115	5.75	16.90	47.15	16.44†	49.45	15.87
116	5.80	17.05	47.56	16.59	49.88	16.01
117	5.85	17.20	47.97	16.73	50.31	16.15
118	5.90	17.35	48.38	16.87	50.74	16.28
119	5.95	17.49	48.79	17.02	51.17	16.42
120	6.00	17.64	49.20	17.16	51.60	16.56
121	6.05	17.79	49.61	17.30	52.03	16.70
122	6.10	17.93	50.02	17.45	52.46	16.84
123	6.15	18.08	50.43	17.59	52.89	16.97
124	6.20	18.23	50.84	17.73	53.32	17.11
125	6.25	18.37	51.25	17.88	53.75	17.25
126	6.30	18.52	51.66	18.02	54.18	17.39
127	6.35	18.67	52.07*	18.16	54.61	17.53
128	6.40*	18.82	52.48	18.30	55.04	17.66
129	6.45	18.96*	52.89	18.45	55.47	17.80

TABLE II—CONT'D

1.	2.	3. MEN	4.	5. WOMEN	6.	7.
PER CENT	RED CELL COUNT, MILLIONS PER C.MM.	HEMOGLOBIN, GRAMS PER 100 C.C.	VOLUME OF PACKED CELLS, C.C. PER 100 C.C.	HEMOGLOBIN, GRAMS PER 100 C.C.	VOLUME OF PACKED CELLS, C.C. PER 100 C.C.	HEMOGLOBIN, GRAMS PER 100 C.C.
130	6.50	19.11	53.30	18.59	55.90	17.94
131	6.55	19.26	53.71	18.73	56.33	18.08
132	6.60	19.40	54.12	18.88	56.76	18.22
133	6.65	19.55	54.53	19.02	57.19	18.35
134	6.70	19.70	54.94	19.16	57.62	18.49
135	6.75	19.84	55.35	19.30	58.05	18.63
136	6.80	19.99	55.76	19.45	58.48	18.77
137	6.85	20.14	56.17	19.59	58.91	18.91
138	6.90	20.29	56.58	19.73	59.34	19.04
139	6.95	20.43	56.99	19.88	59.77	19.18
140	7.00	20.58	57.40	20.02	60.20	19.32
141	7.05	20.73	57.81	20.16	60.63	19.46
142	7.10	20.87	58.22	20.31	61.06	19.60
143	7.15	21.02	58.63	20.45	61.49	19.73
144	7.20	21.17	59.04	20.59	61.92	19.87
145	7.25	21.31	59.45	20.73	62.35	20.01
146	7.30	21.46	59.86	20.88	62.78	20.15
147	7.35	21.61	60.27	21.02	63.21	20.29
148	7.40	21.76	60.68	21.16	63.64	20.42
149	7.45	21.90	61.09	21.31	64.07	20.56
150	7.50	22.05	61.50	21.45	64.50	20.70

The average figures for red cell count, hemoglobin estimation, and volume of packed red cells in the series of healthy men that we recently reported[1] are indicated in the table by ** and the lowest and highest values found are indicated by *. Similarly the averages and extremes of variation found in the study[2] of the bloods of 100 healthy young women are indicated by †† and † respectively.

hemoglobin in index calculations. Hence we use 14.7, the average hemoglobin coefficient for normal men as calculated from the above data, as 100 per cent hemoglobin in calculating indexes for men (column 3 in Table II), and 14.3, the average hemoglobin coefficient similarly calculated for women, as 100 per cent hemoglobin in calculating indexes for women (column 5 in Table II). In like manner we use 41, the average *volume coefficient* (defined by us as the c.c. of packed red cells per 100 c.c. of blood calculated to a red cell count of five million) for normal men, as 100 per cent cell-volume in calculating indexes for men (column 4 in Table II), and 43, the average volume coefficient for normal women, similarly calculated from our data, as 100 per cent cell-volume in computing indexes for women (column 6 in Table II).

The color index expresses the ratio of the hemoglobin per unit number of cells in the patient's blood to the average hemoglobin per unit number of cells in the blood of normal persons of the patient's sex and age group. It is $\frac{\%\text{ hemoglobin}}{\%\text{ red cells}}$. This index is high in uncomplicated pernicious anemia, but low in chlorosis and in anemias due to chronic blood loss, and within normal limits in most other anemias.

The volume index expresses the ratio of the mean size of the cells in the blood examined to the mean size of the cells in the average blood of normal individuals of the patient's sex and age group. It is $\frac{\%\text{ cell-volume}}{\%\text{ red cells}}$. It is high

in all cases of pernicious anemia, and low in anemias due to chronic blood loss and probably also in chlorosis (although this has not yet been sufficiently tested).

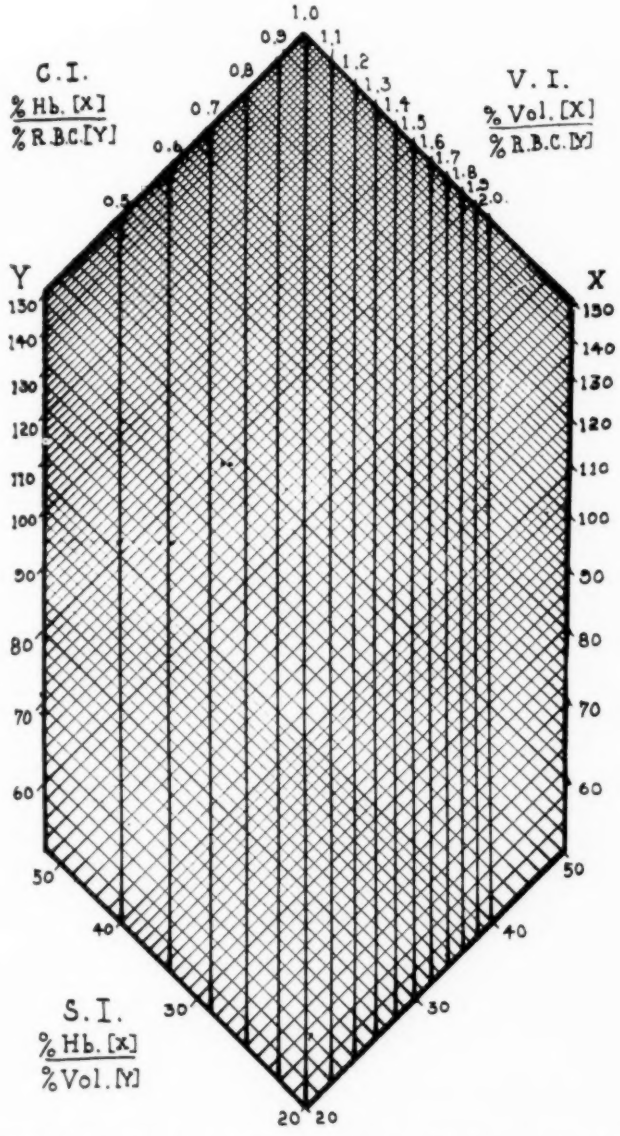

Chart 1.—C.I. = color index. V.I. = volume index. S.I. = saturation index. If either percentage figure is less than 20, double both figures before reading the index from the chart. Indexes of 1.2 or more and 0.8 or less are definitely pathologic.

The saturation index expresses the ratio between the hemoglobin per unit volume of cells in the blood examined and the average hemoglobin per unit volume of cells in the blood of healthy persons of the same sex and in the same age group. It is $\frac{\%\text{ hemoglobin}}{\%\text{ cell-volume}}$. Its significance has not been fully

worked out yet, but apparently it is never high. It is low in anemias due to chronic blood loss even if they complicate pernicious anemia, and is normal in most other anemias including uncomplicated pernicious anemia.

In the cases we have studied 90 per cent of color, volume and saturation indexes in normal individuals have fallen between 0.9 and 1.1 and indexes below 0.8 and over 1.2 have been pathologically significant.

EXPLANATION OF TABLE II

Column 1 is to be read as per cent. The red cell counts in column 2 are arranged on the basis of five million as 100 per cent, so that after finding the patient's count in this column, reference to the corresponding figure in column 1 gives the red cell count expressed as per cent of five million. The hemoglobin figure in column 3 is so calculated that after finding the man's hemoglobin figure expressed in grams per 100 c.c. in this column reference to column 1 will give the hemoglobin expressed as per cent of 14.7 gm., which is the normal hemoglobin coefficient in men.

Column 4 is so calculated that after finding the man's volume of packed red cells expressed as c.c. per 100 c.c. of blood in this column, reference to column 1 will give the cell volume expressed as per cent of 41 c.c., which is the normal volume coefficient in men. The author's technic[1] for cell volume determination must be used.*

Columns 5 and 6 for women are similar to columns 3 and 4 for men but are based on the normal hemoglobin coefficient for women (14.3 gm.) and the normal volume coefficient for women. (43 cc.). Column 7 is inserted for convenience in calculating the grams of hemoglobin in 100 c.c. of blood when the method used for estimation is based on a content of 13.8 gm. per 100 c.c. as 100 per cent.

It is recommended that hemoglobin results be always reported in grams per 100 c.c. because so many different figures have been used as 100 per cent hemoglobin by manufacturers of hemoglobinometers that per cent figures are almost meaningless. We must repeat our warning[3] that, although they are supposedly standardized so that 100 per cent is equivalent to 13.8 gm. of hemoglobin, the Dare and Tallqvist methods are not sufficiently accurate for color index determinations.†

For hemoglobin methods in which 100 per cent is not exactly equivalent to 13.8 gm. per 100 c.c. column 7 must, of course, be recalculated. The number of grams of hemoglobin per 100 c.c. of blood equivalent to an esti-

*This technic in brief is as follows: about 4 c.c. of oxalated (20 mg. powdered potassium oxalate per 10 c.c. of blood) venous blood is centrifugated twenty to thirty minutes at high speed (over 3,000 revolutions per minute) in the special tube described below. The total volume of blood and the volume of cells is then noted and the blood recentrifugated for periods of at least five minutes until the volume of packed cells ceases to change. The c.c. of packed cells per 100 c.c. of blood is then calculated. The special tube is made by sealing the tip of a 10 c.c. Mohr pipette (graduated for 0.1 c.c. to the tip), cutting it off above the 6 c.c. mark (capacity 4 c.c.) and testing it for capacity at each 0.1 c.c. mark. Because of the small diameter of this tube it must be supported as follows: a cork of such diameter that it will rest on the bottom of the metal centrifuge cup is hollowed out to receive the tip of the special tube. A second cork slightly larger in diameter than the metal cup is so cut that it fits partly into it but is prevented by a lip from slipping entirely into the cup. This cork is then bored to fit snugly around the special tube, and so steady it at the top.

†The methods of Van Slyke (J. Biol. Chem. 33:127, 1918), Cohen and Smith (J. Biol. Chem. 39:489, 1919), Haskins (J. Biol. Chem. 57:111, 1923) and Osgood and Haskins (J. Biol. Chem. 57:107, 1923) have been tested and found to be accurate, although only the last three are clinically practical, while the last two have the advantage of utilizing permanent standards. In all of the methods mentioned above an estimation of 100 per cent indicates a content of 13.8 gm. of hemoglobin per 100 c.c. of blood.

mation of 100 per cent with the particular hemoglobinometer used must be placed in column 7 opposite 100 per cent in column 1, and other figures placed to correspond to this value.

EXPLANATION OF THE CHART

Chart 1 is so designed that the vertical line corresponding to the intersection of any two printed lines of the logarithmic paper gives the quotient of the value indicated by the figure in the right-hand column (X) divided by the value indicated by the figure in the left-hand column (Y). Hence, it can be used for the determination of all of the indexes if one simply remembers to always look up the numerator of the fraction expressing the index in the right-hand column (X) and the denominator in the left-hand column (Y).

EXAMPLE OF THE CALCULATION

The study of the blood of Mrs. F. gave the following results:

Red blood cells 1.62 million.

Hemoglobin 52.0 per cent (Haskins-Sahli[4] method).

Volume of packed red cells 18.89 c.c. per 100 c.c. of blood (by the author's technic[1]).

Reference to column 2 (Table II) shows that this red cell count corresponds to 32 per cent (column 1) of 5.0 million red cells.

Reference to columns 1 and 7 shows that an estimation of 52.0 per cent by this method is equivalent to 7.18 gm. of hemoglobin per 100 c.c. of blood.

Then looking up 7.18 in column 5 (the patient is a woman) we find that it is 50 per cent of the normal hemoglobin coefficient for women.

In the same manner looking up 18.89 c.c. in column 6 we find that it is 44 per cent of the normal volume coefficient for women.

Now we can determine the indexes by use of Chart 1. The color index is $\frac{50\ (X)}{32\ (Y)}$*. Therefore, look up line 50 in column X and line 32 in column Y, we find that they intersect about midway between vertical lines 1.5 and 1.6, corresponding to a color index of 1.55.

Similarly the volume index is $\frac{44\ (X)}{32\ (Y)}$. These lines intersect at a vertical line corresponding to a volume of 1.36.

The saturation index is $\frac{44\ (Y)}{50\ (X)}$ which from Chart 1 is found to be 1.14.

The laboratory report on this case would then read:

Red blood cells 1.62 million.

Hemoglobin 7.2 grams.

Color index 1.55.

Volume index 1.36.

Saturation index 1.14.

The high volume and color indexes with normal saturation index is pathognomonic of pernicious anemia.

SUMMARY

Table II in this paper enables one to convert the results of red cell counts, hemoglobin estimations, and cell volume determinations directly into all the percentage figures that are necessary for the calculation of the color, volume and saturation indexes. The table can also be used to convert percentage of

*The letters (X) and (Y) refer to the columns in Chart 1 in which the corresponding numerals should be looked up.

hemoglobin (when estimated by methods in which 100 per cent indicates a content of 13.8 gm. per 100 c.c.) into grams of hemoglobin per 100 c.c. of blood, thus enabling clinicians to report their hemoglobin findings in grams as has recently been strongly recommended.

In addition, the table shows the average figure and the significant variations for red cell counts, hemoglobin estimations, and cell-volume determinations in young men and women.

Chart 1 is so constructed that the color index, volume index and saturation index figures for any blood may be found by inspection without calculation from the percentage figures that have been obtained from Table II.

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[1]Osgood, E. E.: Hemoglobin, Color Index, Saturation Index, and Volume Index Standards. Arch. Int. Med., May, 1926, xxxvii, 685.
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[3]Haskins, H. D.: A New Permanent Standard for Sahli's Hemoglobinometer, Jour. Biol. September, 1926, xxv, 500.
[4]Haskins, H. D.: A New Permanent Standard for Sahli's Hemoglobinometer, Jour. Biol. Chem., August, 1923, iii, 57.

A METHOD FOR CAPILLARY BLOOD SUGAR*

By H. V. Gibson, M.D., Madison, Wisconsin

THERE have recently been published a number of capillary blood-sugar methods but we have been unable to use any of them satisfactorily in clinical work because of the quantity of blood required, special apparatus needed, or inaccurate results obtained. The following method represents the technic I adopted after considerable experimentation. It requires no unusual apparatus and involves but one micro measurement—that of the original blood sample. The maximum error found for the method has been 3 per cent when checked independently in three separate laboratories.† The method is a modification of the widely used Folin-Wu technic.[1]

THE METHOD

Apparatus.—1. A capillary pipette graduated to contain 0.1 c.c.

2. Serology test tubes, of 4 c.c. capacity with stoppers, one tube for each sample.

3. Folin-Wu sugar tubes, calibrated at 10 c.c.

4. Lancet for capillary puncture.

Solutions.—1. 0.115 N H_2SO_4.

2. 3⅓ per cent sodium tungstate.

*From the Laboratories of the Wisconsin Psychiatric Institute, University of Wisconsin and the Obstetric Department, Washington University School of Medicine, St. Louis, Mo.
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†Chemistry Laboratory of Wisconsin General Hospital, Biochemistry Department Laboratory, and the author's laboratory, all of University of Wisconsin.

3. Alkaline copper and phospho-molybdate solution for Folin-Wu method.

4. Water, alcohol and ether for cleaning and drying pipette.

5. Standards = 0.04 and 0.05 mg. glucose per c.c. in saturated benzoic acid solution.

Procedure.—As many 4 c.c. tubes as the number of samples expected are racked, 1.9 c.c. of 0.115 N H_2SO_4 placed in each, and the lot stoppered. Next, 0.1 c.c. blood is obtained from a puncture wound of the ear or finger tip and rinsed into the 0.115 N H_2SO_4 by alternately sucking up and blowing out. The tube is then stoppered, inverted to mix and set aside until the experiment is over. When all the tubes are so prepared, 1 c.c. of 3⅓% sodium tungstate solution is added to each. The tube is then stoppered, shaken and centrifuged at high speed for ten minutes. Two c.c. of the supernatant fluid are then pipetted off and placed in one of the Folin-Wu sugar tubes. Standards containing 0.8 and 0.1 mg. glucose in 2 c.c. are suitable for all ordinary work. (Such standards keep for weeks if made up in half saturated benzoic acid solutions.)

The alkaline copper reagent and the digestion and color development carried out exactly as in the macro-Folin-Wu method. The final dilution is made to 10 c.c.

With standard set at 20 and X = reading of the unknown; for the 0.08 mg. standard $\frac{2400}{X}$ = mg. glucose per 100 c.c. of blood and for the 0.1 mg. standard $\frac{3000}{X}$ mg. glucose per 100 c.c. of blood.

Comparison with the macro-Folin-Wu sugar method[1] on the same samples were as follows:

Mg. Sugar per 100 c.c. of Blood

SAMPLE	MACRO	MICRO	DIFFERENCE
1	179.6	178	0.9 %
		178	
2	109.9	112.1	2.0 %
		111.7	1.6+%
3	106.5	106.8	0.2+%
	105.7	105.2	0.4+%

REFERENCE

[1]Folin, O., and Wu, H.: Jour. Biol. Chem., 1920, xli, 367.

METHODS FOR MAKING A STABLE EMULSIFIED SYPHILITIC ANTIGEN*

By Frederick Proescher, M.D., Albert Arkush, A.B., and Albert Krueger, Agnew, California

ALCOHOLIC heart extracts, plain or fortified with cholesterin and acetone-insoluble lipoids of normal tissue, are at present the antigens of choice for the syphilitic complement-fixation test.

Ethyl alcohol 96 per cent (or absolute), methyl or propyl alcohol has been found so far to be the best extraction medium. In order to obtain a good antigen the wet or dried tissue should be extracted at least five to eight days or even longer at 38° C. to secure the maximum of antigenic activity.

For several years Dr. Proescher has conducted a large number of experiments to find another extraction medium which shortens the extraction time and removes the maximum of the specific lipoids without removing the anti-complementary and hemolytic substances.

The following fat solvents were used: chloroform, carbontetrachlorid, carbonbisulphide, pyridin and petrol ether. Chloroform gave the best results either with wet or dried tissue; almost equally as good was pyridin, but on account of its disagreeable odor this was discarded.

The alcoholic extracts must be carefully diluted with saline solution in order to obtain a suitable antigen. The state of dispersion greatly influences the antigenic properties. Saline diluted alcoholic extracts are unstable. The advantage of a stable emulsified antigen is obvious.

The original antigen was prepared from fresh beef hearts by direct extraction with chloroform. This antigen will be designated as "Chloroform Antigen I" and contains the neutral fats plus the specific lipoids (Lecithin, Phosphatides) and a natural amount of cholesterin (0.1 to 0.13 per cent). Since the neutral fats are without antigenic properties and make the final emulsification somewhat difficult, we have lately used dried beef heart and the neutral fats were completely removed by petrol ether,† previous to the chloroform extraction. This new antigen will be designated in the following as "Chloroform Antigen II"; it does not contain cholesterin.

PREPARATION OF "CHLOROFORM ANTIGEN I"

To 500 gm. of fresh, preferably not chilled, finely ground beef heart add 250 c.c. chloroform‡ and agitate for twenty-four hours in the shaking ap-

*From the Pathological Laboratory of Agnew State Hospital.

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†The average neutral fat content of 9 dried beef hearts was 11.1 per cent, the lowest was 8.58 per cent, the highest 14.45 per cent. The average chloroform soluble lipoid content of 7 beef hearts, extracted at room temperature, for twenty-four hours previously extracted with ether, was 3.08 per cent; the lowest was 2.84 per cent, the highest 3.23 per cent. The water content of 7 dried beef hearts was in the average 5.2 per cent, the lowest 4.05 per cent, the highest 5.95 per cent. The ether extract contained 0.15 to 0.2 per cent cholesterin.

‡The chloroform should be acid free and preferably distilled before use.

paratus at 37° C.* Decant the chloroform off by draining the bottle over a funnel. The chloroform-lipoid solution is then filtered repeatedly through a thick, dry filter paper until perfectly clear.

The filtrate, which is usually of a golden yellow color, is poured into a crystallizing dish and the chloroform evaporated with the aid of a fan at room temperature. The chloroform should be entirely evaporated by stirring the fatty material with a glass rod, until the last traces of chloroform are driven off. Add to the lipoid material, which is of a creamy consistency, about 50 gm. washed and ignited sea sand and 10 c.c. redistilled 96 per cent alcohol. Mix thoroughly and transfer into a clean, heavy walled, narrow mouthed bottle, and add 200 c.c. of normal saline solution containing 0.3 per cent carbolic acid. The carbolic acid saline solution should be slowly added with vigorous shaking. For proper emulsification the bottle should be agitated in a shaking apparatus for an hour. The resulting milky emulsion is finally filtered through a loose cotton plug to remove the sea sand and unemulsified lipoids.

We usually extract portions of 4 or 5 beef hearts, separately at the same time, since not every heart gives a satisfactory antigen. Experience has shown that if the chloroform soluble lipoids do not emulsify, and only a slightly opalescent but not milky emulsion is obtained, its antigenic properties are too weak to be used. About 40 per cent of the hearts will give a satisfactory antigen.

PREPARATION OF "CHLOROFORM ANTIGEN II"

Fresh beef heart is finely ground in a meat grinder, spread out in a thin layer on glass plates and dried† at 37° C. in the incubator with the aid of a fan. The dried muscle is ground again in a mill until a fine powder is obtained. If not perfectly dry, it is spread out in a thin layer on glass plates and dried for several days at 37° C. The dried powder is stored in amber colored bottles and will keep for several months. Fifty to 100 gr. of powdered beef muscle is filled in a paper extraction thimble and for three days extracted in the Soxleth Extraction Apparatus with petrol ether. After a complete extraction the contents of the thimble are spread out on a glass plate and dried, until the ether is completely evaporated. The ether removes, besides the neutral fats and cholesterin, the fatty acids. The ether free heart muscle is filled in a narrow necked amber colored glass bottle and 100 to 200 c.c. redistilled chloroform added and agitated for twenty-four hours at room temperature (or incubator 37° C.) in the shaking apparatus. The chloroform extract is filtered through a thick filter paper and the clear chloroform solution poured in a crystallization dish, the chloroform completely evaporated and the remaining lipoids emulsified in a 100 to 150 c.c. phenolized saline solution as described above.

*If a shaking apparatus is not available the extraction may be accomplished in the incubator with occasional shaking.

†For rapid drying the ground heart muscle (500 gm.) is mixed in a large beaker with 1000 c.c. acetone and let stand for an hour with occasional stirring. It is filtered through a Buechner funnel, spread on glass plates and dried at 37° C. The acetone acts as a dehydrating agent and removes some of the neutral fats without removing the specific lipoids. In this way large amounts of ground heart muscle can be dried in a short time without the aid of a fan.

If desired the lipoids may be kept in a vacuum desiccator over concentrated sulphuric acid or phosphoric anhydride. If protected from light and kept at ice box temperature they will keep indefinitely.

Lately we have evaporated the chloroform extract in large thick walled test tubes (20 × 2½ cm.) and after complete evaporation of the chloroform, the remaining lipoids are dried in a vacuum desiccator over phosphoric anhydride. After a week's drying, the end of the test tube is drawn out into a small tube and connected with the vacuum pump and evacuated as completely as possible and sealed while still in connection with the vacuum pump. The sealed tubes are stored in the refrigerator.

If an aliquot part of the original chloroform extract is evaporated, emulsified and titrated against a polytropic syphilitic serum, measured amounts of the chloroform extract may be evaporated in a large test tube, dried, sealed, and for use a definite amount of phenolized saline solution added.

In this way a standardized amount of lipoids can be kept on hand ready for immediate use.

PROPERTIES OF EXTRACTS

The antigenic, anticomplementary and hemolytic properties of the chloroform-soluble lipoids compare favorably with alcoholic heart extract fortified with cholesterin and the acetone insoluble lipoids of normal tissue.

An ideal extract should be highly antigenic and as little hemolytic and anticomplementary as possible. If used in doses of two to four antigenic units, its anticomplementary and hemolytic properties should be at least ten times less than its antigenic properties. The antigenic activity of a good "Chloroform Antigen I" should be at least 0.05 c.c. if titrated against 0.1 c.c. of a mixture of several syphilitic sera using 2 or 3 units of amboceptor and 3 or 4 units of complement. Very good antigens may protect in doses of 0.005 to 0.001 c.c.

With the Method II we were able to obtain in 100 per cent of the hearts a suitable antigen of even higher potency than with the original method.

The following table gives the titer of 14 different antigens* titered as above:

EXTRACT	AMOUNT OF EXTRACT GIVING COMPLETE INHIBITION c. c.
1	0.004
2	0.001
3	0.0001
4	0.0001
5	0.002
6	0.0001
7	0.001
8	0.0002
9	0.0001
10	0.001
11	0.006
12	0.001
13	0.0002
14	0.0006

*Chloroform soluble lipoids of 9 gr. of dried beef heart emulsified in 15 c.c. saline.

All of the above antigens were neither anticomplementary nor hemolytic in doses of 1 c.c. of the undiluted antigen.

Kolmer has called attention to the fact that a good antigen should be highly polytropic or, in other words, that it should have affinity for the lipodophilic antibodies in the sera of all syphilities. The chloroform antigen seems to be slightly more sensitive than the fortified alcoholic heart extract, if the ice box incubation is used. In 104 cases of paresis the blood Wassermann was positive in 97 per cent with the chloroform antigen and in 95 per cent with the cholesterinized alcoholic beef heart. In untreated cases of secondary syphilis results were positive in 98 per cent of the cases.

The advantage of the chloroform antigen is the great stability of its antigenic properties without becoming anticomplementary or hemolytic. We have so far never encountered an extract which would not keep at least for one year, while alcoholic heart extracts may become anticomplementary in a few weeks.

A NOTE ON THE MEASUREMENT OF BLOOD FOR CHEMICAL EXAMINATION*

By S. L. Leiboff, A.M., New York, N. Y.

WHILE we pay a great deal of attention to the refinement of technic in the chemical examination of blood, we usually overlook one important factor which introduces a source of error, and that is the accurate measurement of the blood.

The assumption that the pipettes at our disposal are quite accurate is an erroneous one; thus, when I tested a dozen Folin blood pipettes, picked at random, for their accuracy, I found great variations in the amounts of blood delivered by the various pipettes. The blood was drawn into each pipette from a flask containing citrated sheep blood, to the 5 c.c. mark, and weighed in stoppered weighing bottles, thus avoiding loss by evaporation. The blood was shaken well each time in order to avoid variations in the proportion of cells to plasma; and at no time was any sample of blood, after being withdrawn, returned to the flask, since during the transfer the proportion of cells would diminish; the cells being more viscous than the plasma, more would stick to the sides of the pipette and weighing bottle, thus introducing a source of error. The largest variation in the weights of the blood delivered by the different pipettes was 4.6 per cent.

Another source of error is introduced by the inequality in the diameter of the tip in different pipettes, since the greater the diameter of the tip the faster the blood will flow, thereby leaving a greater amount of blood stuck to the walls of the pipette, particularly since blood is a rather viscous fluid.

Still another error is introduced by the variations in the viscosity of the

*From the Biochemical Department of Lebanon Hospital Laboratory.
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blood in various diseases. The viscosity of a liquid is the resistance it offers to flowing or changing its shape, since in a liquid the size of the molecules relative to the space between them is so large that the molecules get into each other's way, thus limiting their freedom of movement. Accordingly, the higher the viscosity of the blood the more of it will stick to the sides of the pipette, thereby delivering a lesser amount. The great viscosity of the blood is due chiefly to the corpuscles, but also to the proteins by reason of their being hydrophilic colloids.

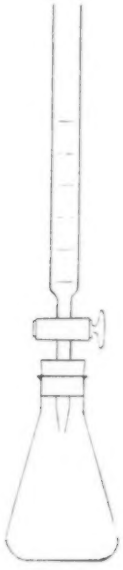

Fig. 1.

The simple device shown in the picture is recommended to overcome these difficulties. It is a short burette with a glass stopcock, about 15 mm. in diameter. It can easily be standardized to a high degree of accuracy by placing into it 13.5585 gm. of pure mercury for every c.c. of blood and marking the glass at the meniscus of the mercury, this being the weight of 1 c.c. of mercury at 15° C. which is the usual laboratory temperature.

To measure out blood, the burette, with the stopcock closed, is filled with the desired amount of blood, taking care not to introduce any blood above the mark. The stopcock is then opened and the blood clinging to the burette is washed down with the amount of water required for the dilution of the blood.

A PORTABLE THERMOELECTRIC APPARATUS FOR THE DETERMINATION OF SURFACE AND TISSUE TEMPERATURES*

By H. C. Bazett, M.D., and B. McGlone, Ph.D., Philadelphia, Pa.

THERMOELECTRIC determinations of the temperature of human tissues were reported by Becquerel and Breschet (1835), and this method has been employed in numerous subsequent investigations. As shown by these authors (1839) many of the obvious difficulties attendant upon the use of mercury thermometers are eliminated, and the errors common to both methods are reduced when thermocouples are employed. Benedict (1925) in discussing the temperatures of the surface of the skin has also pointed out the advantage of thermocouples of small dimensions capable of close contact as compared with the relatively large bulb of mercury instruments. To adapt the cumbersome laboratory equipment to clinical use and to maintain a high degree of accuracy this portable thermoelectric apparatus has been constructed.

In Fig. 1 (X 1⅕), it will be noted that the apparatus consists of two thermocouples (*S* and *N*), a constant temperature bath (*B*), and galvanometer (*G*). The surface thermocouple (*S*) is of the type suggested by Benedict (1925), employing, however, as thermoelements constantan and iron, and wire of smaller diameter (0.32 mm.). The constantan wire leads from the variable thermojunction (at *S*) to the constant junction within the bath (*B*), a length of about 130 cm., entering the bath by a lead (*t*); at this point an iron wire is soldered, forming the constant thermojunction, and this wire is continued as one of the galvanometer leads (*a*). The iron thermoelement from the variable junction leads directly to the galvanometer by lead (*e*). The wires are silk insulated, and to secure further insulation and rigidity they are enclosed in rubber tubing (inside diameter, 8.0 mm., thickness of wall, 2.0 mm.). At the exits from this larger tubing each wire is enclosed singly in tubing of thinner wall, and of 5.0 mm. external diameter. The total resistance of this thermocouple and its leads should be about 11.0 ohms and in the example described is 11.8 ohms.

A steel constantan thermocouple of needle type which is a modified form of the design of Lefèvre (1898, 1911) is employed for the determination of dermal and subcutaneous temperatures. Fig. 2 shows the construction of this needle thermocouple. Steel tubing (*1*) as drawn for hypodermic needles 0.45 mm. diameter, of 14.5 cm. total length, is supported for 8.7 cm. of its length in a hard rubber cylinder (*2*); the needle is fixed at both ends of the cylinder by plaster of Paris, leaving between these points of fixation an insulating airspace. The steel tubing protrudes from the hard rubber support a length of 5.8 cm. and is protected by a cylindrical cover of brass (*3*) 8.3 cm. in length

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and of 1.4 cm. external diameter; as the thickness of the brass wall is about 0.3 cm. a considerable air space is obtained. This brass cover is continuous below with a hard rubber cone (*1*) of 1.1 cm. length and support is thus given to the needle, which touches the hard rubber cone at the exit of the needle for a length of 1.0 mm. The hard rubber cylinder (*2*) has a screw thread (pitch usually 1 mm.); the brass cover (*3*) has a detachable screw head (*5*) which fits the thread of the hard rubber cylinder (*2*). Thus the brass cover may be adjusted to give a variable length of needle beyond the tip of the hard rubber cone, and by reference to the number of turns of the cover (*3*), the distance exposed, and so the depth to which the thermojunction is buried

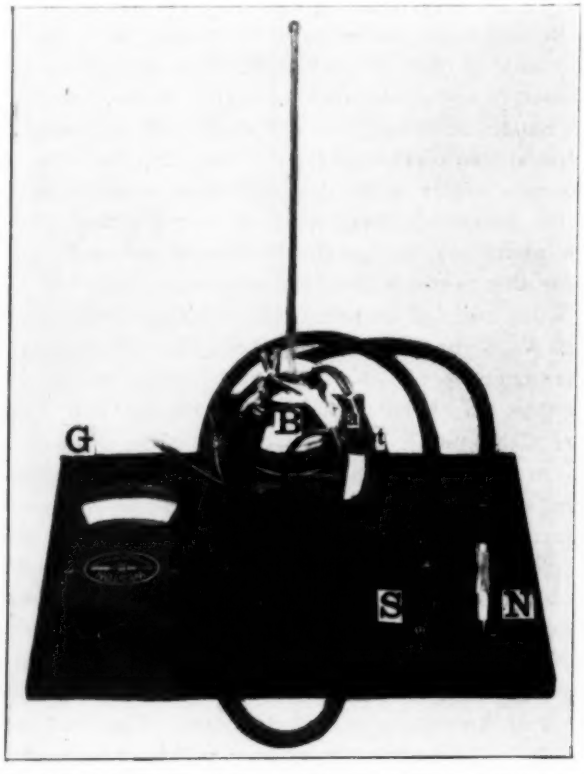

Fig. 1.—Portable thermoelectric apparatus (X⅕). Description in text.

in the tissue can be ascertained. At the upper end of the steel tubing, a lead of similar steel tubing (of 0.36 mm. diameter) is soldered, and without further break, this leads to the galvanometer (Fig. 1, *G*). Through the steel tubing in the needle proper insulated constantan wire (0.127 mm. diameter) is threaded, and soldered to the steel at the beveled end of the tubing (Fig. 2, *J*); this constantan wire is continued without break to the constant temperature thermojunction in the bath, which junction is made by soldering to the constantan steel tubing which in turn serves as the galvanometer lead. The wires are enclosed and supported in rubber tubing as previously described. In this case the leads were made up of two strands of steel tubing and suffi-

cient strands of constantan so as to reduce the total resistance to 12.9 ohms. The galvanometer leads of both surface and needle thermocouples are readily detached from the binding posts of the galvanometer to facilitate rapid substitution.

The constant temperature bath (Fig. 1, *B*) consists of a thermos flask of one pint capacity, containing paraffin oil, and sealed with cork. Through perforations in the cork the constant temperature thermocouple leads enter the bath, and in addition the cork supports a thermometer, so adjusted that its bulb is at the level of the thermojunctions.

The galvanometer is of the double pivoted movable coil type (Weston, model 440), of an internal resistance of 3.5 ohms, a period of 2.3 seconds, and a deflection indicating 2.2 microamps per division.

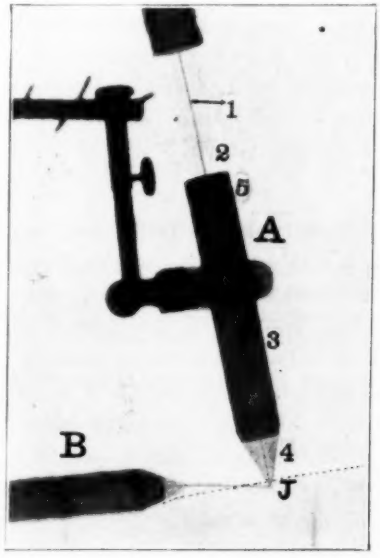

Fig. 2.—X-ray photograph of thermocouple needles (X½). Description in text. Thermocouple *A* is inserted radially and *B* obliquely into the subject's forearm. The dotted line indicates the margin of the skin.

As can be seen in Fig. 1, both the galvanometer and constant temperature bath are secured to a baseboard, and spring clamps support the thermocouples when not in use.

The constant temperature bath is kept at room temperature. To determine the "temperature factor" or value in degrees of temperature per scale division the thermocouples should be standardized against a mercury thermometer in water, perferably at least once during each experimental day, and the standardizing temperatures, two or more, should if possible be higher than that of the constant bath, unless the environmental temperature should by chance be higher than that of the surface or tissue studied.

Due care should be observed to prevent rusting, and the exposed portions must after using or standardizing be dried and coated with a pure vaseline. The mercury thermometers employed in this set are about 40.0 cm. in length and graduated from –10.0° to 50.0° C. in $\frac{1}{10}$°.

The sensitivity of the set illustrated is between 0.6° and 0.8° C. per scale division, insuring an accuracy of at least 0.2° C. in the estimation of the thermocouple temperature. If the needle be inserted obliquely into the tissues to a length of 10 mm. or more (as illustrated in Fig. 2, needle *B*), the thermocouple causes only slight thermal disturbances in the tissues and the temperature so determined may be considered as that of the tissue. If on the other hand the needle be vertically inserted to a small depth (less than 5 mm., as needle *A*, Fig. 2), the temperature gradients are modified by the presence of the needle and the temperatures calculated are considerably lower than those normally existing in the tissues. At room temperatures of 22° or 23° the calculated temperatures are approximately too low by 0.5° for a depth of 3 mm., 0.3° for 5 mm., and 0.2° for 7 mm. The actual depth of the thermal junction in obliquely inserted needles can be estimated from a consideration of the angle of insertion. Needle thermocouples have been described by Adrian and Watts (1923), and have been employed by Lewis and Love (1926). Needles of the type described by the former authors are less accurate than those here described, since complicating factors are introduced by the use of an ordinary hypodermic needle, which is nickel plated and, in addition, usually has a brass seating.

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A COLORIMETRIC METHOD FOR THE DETERMINATION OF IRON AND HEMOGLOBIN IN THE BLOOD*

By Martin Dupray, B.S., M.S., Hutchinson, Kansas

COLORIMETRIC BLOOD IRON METHOD

Wong[1] has described a colorimetric method for the determination of the iron in the blood. In his method the organic matter of the blood is destroyed by sulphuric acid and potassium chlorate, and the iron estimated as ferric thiocyanate.

The destruction of the organic matter in Wong's method requires immediate attention throughout the process as the boiling of the acid must be interrupted several times to add fresh potassium chlorate, and this addition must be made with some care with the tube inclined to avoid spattering or even explosion.

I have found that perchloric acid is much superior to potassium chlorate in this digestion. The proper amount of perchloric acid may be added to the sulphuric acid to bring about complete digestion in ten or twelve minutes of boiling, and with rare exceptions, enough of the perchloric acid persists throughout this boiling to complete the digestion without further additions. The oxidation, while rapid, is not violent. A little nitric acid included in the digestion mixture materially hastens the early stage of the digestion and shortens the total time a little. With the digestion mixture to be described, digestion is complete in from seven to ten minutes, with very little attention required.

Wong uses a standard ferric iron solution prepared by oxidizing a ferrous ammonium sulphate solution to the ferric state with potassium permanganate. When this solution stands some time a brown sediment, probably manganese dioxide, appears. In the original oxidation manganese is reduced from the heptavalent to the divalent state and remains in solution as manganous sulphate. Its precipitation as the dioxide indicates a reversion to the quadrivalent state at the expense of the iron, part of which is reduced to the ferrous state. This necessitates frequent additions of small amounts of permanganate to maintain a pink color indicating complete oxidation before using the standard, which in time dilutes the standard. Also the precipitate of manganese carries down traces of iron with it. I once found three milligrams of iron in the sediment formed in one liter of standard solution prepared by Wong's method, some two months after preparation. This introduced an error of three per cent in the standard.

A more accurate standard can be prepared by using metallic iron as the starting point. Finely drawn iron wire of known purity is readily obtain-

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able from the supply houses, with the true iron content stamped on the packages. When this is dissolved in a moderate excess of *hot nitric* acid the resulting solution is ferric iron and remains so, and the above errors are avoided. (The use of a cold dilute nitric acid, or of sulphuric acid would yield a ferrous iron solution.)

In Wong's method the ferric thiocyanate color fades rather rapidly, probably from reduction by sulphuric acid. Also the tests fade less uniformly than the standards, probably due to varying amounts of chlorine in the digests. This makes necessary the addition of the thiocyanate to the test and standard simultaneously and reading of the colors at once. Even then, some errors, due to fading, are unavoidable. A little nitric acid added before developing the color prevents this fading. By using this procedure, standards and tests four hours old show no fading when compared with a freshly prepared standard.

These facts have been incorporated into the following method.

REAGENTS REQUIRED

1. *Digestion Mixture.* Twenty-five c.c. of distilled water are placed in a small flask and 50 c.c. of iron-free sulphuric acid, specific gravity 1.84, are added slowly while stirring. The solution is cooled, after which are added 15 c.c. of 60 per cent perchloric acid, and 10 c.c. of iron-free nitric acid, specific gravity 1.42, and the solution mixed and transferred to a glass-stoppered bottle.

2. *Potassium Thiocyanate Solution.* The same as used by Wong, approximately 3 N, or 29.2 gm. of potassium thiocyanate per 100 c.c. of solution.

3. *Standard Ferric Iron Solution.* A portion of finely drawn iron wire of known iron content is thoroughly cleaned with fine emery cloth or paper, until certain of the absence of rust and dirt. A sufficient quantity to give 100 mg. of iron is accurately weighed. For example, if the wire contains 99.8 per cent of iron, 100.2 mg. of the wire is weighed out. Ten c.c. of iron-free nitric acid, specific gravity 1.42, and about 40 c.c. of distilled water are placed in a small flask and brought to a boil. The wire is rolled into a loose coil and dropped into the boiling acid, together with any trimmings used in making the exact weight. Solution is complete in a few moments. The solution is boiled for about one minute after solution appears complete, then cooled. It is then transferred without loss to a 1000 c.c. volumetric flask, the small flask rinsed with several portions of distilled water and the rinsings added to the solution in the volumetric flask. Finally the solution is made up to 1000 c.c. with distilled water and mixed. Each cubic centimeter contains 0.1 mg. of ferric iron. The solution is permanent.

METHOD

One c.c. of well mixed oxalated blood is transferred to a test tube containing exactly 4 c.c. of distilled water, using an Ostwald-Folin pipette, preferably one graduated to deliver between marks. The diluted specimen is mixed and allowed to lake, after which it is mixed again, and 1 c.c. of the

diluted sample placed in a large pyrex test tube, 25 mm. by 200 mm., with a graduation at 25 c.c. One c.c. of the digestion mixture and two or three glass beads* are now added, and the tube placed in a holder over a micro burner. The contents are boiled gently until digestion is complete, which usually requires from seven to ten minutes. Most of the action takes place after the water is boiled off and white fumes appear. Very rarely a blood is encountered, usually one high in fats, which will not completely digest. If it is obvious, after twelve minutes or so of boiling, that digestion will not go to completion, remove the flame and allow the tube to cool slightly. Then add one or two drops of 60 per cent perchloric acid, and resume the digestion. A very little more boiling will then suffice. When digestion is complete the fire is removed and the tube cooled. The liquid will not usually be entirely colorless, when hot a more or less yellow color persisting, due to chlorine and nitrous fumes. This color fades on cooling, leaving a colorless liquid when cold. The tube may be allowed to cool to room temperature before adding water.

When cold, two or three drops of strong nitric acid are added to insure an excess, as the nitric acid in the digestion mixture may be all boiled out. The volume is then brought to about 15 c.c. with distilled water, and 5 c.c. of the thiocyanate solution are added, after which the volume is carefully brought to 25 c.c. with distilled water, and the solution mixed. It is then ready to compare in the colorimeter with the standard.

The standard is prepared by placing 1 c.c. of the standard ferric iron solution (containing 0.1 mg. of ferric iron) and 1 c.c. of the digestion acid mixture in a similar large test tube marked at 25 c.c. Distilled water is added to about 15 c.c., 5 c.c. of the thiocyanate solution are added, and the volume brought carefully to 25 c.c. with distilled water and mixed. There is sufficient nitric acid in the digestion mixture that no more need be added. Check the colorimeter cups against each other with the standard color solution. Then set the standard at 20 mm.

Calculation.

$$\frac{\text{Reading of standard in mm.}}{\text{Reading of unknown in mm.}} \times 50 = \text{Milligrams of iron per 100 c.c. of blood.}$$

Using 0.335[3] as the percentage of iron in hemoglobin,

$$\frac{\text{Milligrams of iron per 100 c.c. of blood}}{3.35} = \text{grams of hemoglobin per 100 c.c. of blood.}$$

The standard may be varied in strength so as to make the calculation direct in per cent of hemoglobin as related to some normal standard. For example, taking Haden's[4] figure of 15.6 gm. of hemoglobin per 100 c.c. of blood as normal, and 0.335 as the per cent of iron in hemoglobin, the iron content of the blood at this normal becomes 52.26 mg. of iron per 100 c.c. of blood. If then the standard ferric iron solution is made to contain 104.5 mg. of iron per

*Folin and Wu[2] pointed out that the antibumping properties of glass beads and quartz pebbles came from the presence of air in the fine pores. Following up this idea it was found that one could obtain at a department store small glass beads known as "satin" beads, which derive their satiny or frosted appearance from the presence of numerous small bubbles and strize in the glass. These beads have proved unusually efficient in preventing bumping.

liter instead of 100 mg., and 1 c.c. be used in the standard color tube, the computation becomes,

$$\frac{\text{Reading of standard in mm.}}{\text{Reading of unknown in mm.}} \times 100 = \text{hemoglobin in per cent of Haden's normal.}$$

SUMMARY

A method for the quantitative colorimetric estimation of the iron, and from it the hemoglobin of the blood is described. It is essentially a modification of Wong's method, but is somewhat less tedious, and avoids some of the errors of Wong's method.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PEPTIC ULCER: Etiology and Pathology of Peptic Ulcer, Levine, S. Am. Jour. Med. Sc., July, 1926, clxxii, No. 1, p. 22.

From a survey of available information concerning peptic ulcer Levine thus summarizes the present status of knowledge of this condition:

Peptic ulcer starts as an erosion.

Erosions may be produced by mechanical, chemical, or physical causes, circulatory and nervous disturbances, infection, and allergic phenomena.

Erosions in the region of the Magenstrasse do not heal readily because of its peculiar structure.

Erosions are converted into peptic ulcers by the destructive action of the acid gastric juice.

The isthmus, pyloric canal, and first portion of the duodenum, because of the resistance they offer to the acid chyme, are particularly liable to be involved in ulcerative processes.

HEMORRHAGE: Hemorrhages, Their Significance and Methods of Treatment, Mills, C. A. Am. Jour. Med. Sc., July, 1926, clxxii, No. 1, p. 71.

In a scholarly review of hemorrhage which well repays reading in the original Mills recalls the efficacy of anaphylactic reactions as a means of treatment in hemophilia. The patient is sensitized by the subcutaneous injection of 4 c.c. of sheep or hen serum and a reaction provoked seven to ten days later by the intracutaneous injection of a few drops. If a single reaction does not bring the clotting time to normal, the reaction may be repeated at weekly intervals.

The injection of the mother's whole blood into the superior longitudinal sinus is an efficient method of treatment in hemorrhage of the newborn.

Mills looks forward to the discovery of the mechanism by which hemorrhage stimulates resistance to disease and to the intelligent use of bleeding as a therapeutic measure.

PERNICIOUS ANEMIA: Etiology and Treatment of Pernicious Anemia, Barker, L. F. Jour. Am. Med. Assn., July 10, 1926, lxxxvii, 80.

Granting that it is literally true that neither the cause of nor an effective treatment for this disease is as yet known, Barker summarizes the advances and additions made in the knowledge of the disease in recent years as follows:

1. Though the causes of pernicious anemia are not yet fully known, clues to their nature are being obtained.

2. In any consideration of etiology, due attention should be paid to the peculiarities of incidence and distribution of the disease; to the fact that it is predominantly a malady of middle and later life; to the characteristic features of the blood picture and their relations to blood destruction within phagocytes and to blood regeneration of embryonal type; to the associated disturbances of the digestive, nervous and endocrine systems; to certain special marks in the bodily configuration; to the occurrence of spontaneous and of therapeutically inducible remissions of variable duration, and to the inevitability, in the present state of knowledge, of a fatal termination.

3. Of the many conceptions of etiology that have been advanced, the evidence at present favors hereditary (genotypic) predisposition as the main factor and various influences in the external conditions (especially poisons derived from bacteria, fungi or animal parasites in the digestive tract) as accessory releasing or provocative factors.

4. Parallel with the growth of hypotheses of etiology, conceptions of pathogenesis are being extended; the causes of the disturbances of equilibrium between blood destruction and blood regeneration (and their antecedents) are becoming clearer; the anemia is recognized as only one part of a comprehensive disease entity in which the digestive system, the nervous system and the endocrine system are also involved, and investigators are now striving to establish correlatives among the various phenomena observable and to find the precise place in the malady as a whole that should be assigned to each integral part.

5. Treatment of the disease, though not curative, is rewarding. Through rest, the administration of dilute hydrochloric acid and of arsenic, injections of blood and other measures, the patient's condition can often be greatly ameliorated, and in many instances remissions of variable duration may be induced. Early recognition of achylia, parethesias, glossitis, and of megalocytosis (before anemia develops) may permit prompt treatment that will tend to keep the malady latent. Intermarriage of members of families in which the disease is known to occur should be discouraged.

The paper contains a large amount of data succinctly expressed and should be read in the original.

PERNICIOUS ANEMIA: Blood Changes in Rabbits Resembling Those in Pernicious Anemia Accompanying B. Welchii Infections, Reed, G. B., Orr, H. J., and Burleigh, C. H. Can. Med. Assn. Jour., May, 1926, xvi, No. 5, p. 525.

It has been shown that a highly virulent strain of B. welchii may produce chronic or acute infection in rabbits depending upon the age of the culture used.

Rabbits suffering from acute or chronic infection with B. welchii develop a profound anemia characterized by a decrease in red cell numbers without a corresponding decrease in hemoglobin and by conspicuous anisocytosis.

Quantitative examination of the degree of anisocytosis shows that it resembles that of pernicious anemia in man.

These results are presented in the form of a progress report, other data are in process of publication elsewhere concerning the action of B. welchii toxin on red blood cells both in vivo and in vitro and on other tissues.

URIC ACID: Uric Acid and Creatinine in the Urine of Infants, Rougichitch, O. S. Am. Jour. Dis. Child., April, 1926, xxxi, No. 4, p. 505.

The uric acid excretion per twenty-four hours was measured in a series of twenty-two male infants. The diet of the infants being purine free, the amounts of uric acid found are regarded as of endogenous origin. In contrast with most of the hitherto published measurements of uric acid in infants' urine, a closely constant daily excretion was found for the individual, and a fairly constant value per unit of body weight for the group of infants, the range being from 14 to 25 mg. of uric acid per kilogram of body weight.

Except in the case of an infant with active rickets whose uric acid excretion was high, 36 and 34 mg. per kilogram of body weight, no relationship of nutritional state to uric acid excretion was apparent. Uric acid excretion was also apparently independent of the age of the infant.

It is suggested that the relatively high level of excretion of endogenous uric acid by infants may be explained by their high protein intake; using the evidence obtained by Folin and his coworkers that a high intake of protein retards the destruction and accelerates the excretion of uric acid. It must be admitted, however, that in this series the relationship of urine nitrogen to uric acid was not close, uric acid nitrogen ranging from 1.25 to 3.5 per cent of the total nitrogen.

Measurements of urine creatinine in the same group of infants support the conception of a close constancy of creatinine excretion in terms of the protoplasmic mass of the body. The range found was from 10 to 15 mg. per kilogram of body weight. The highest values were obtained in malnourished infants, probably because of their relatively larger mass of active tissue.

No relationship of creatinine excretion to differences in sleeping time or of muscular

tonus was discernible. In the case of an infant having severe generalized convulsions, however, a creatinine excretion three to four times higher than normal was observed. Another infant with slight localized convulsions was found to excrete an approximately normal quantity of creatinine.

MERCUROCHROME: The Present Status of Mercurochrome—220 Soluble, Davis, H. B. Am. Jour. Med. Sc., September, 1926, clxxii, No. 6, p. 340.

There have been so many conflicting reports concerning this substance, many so biased or lacking in controls as to be difficult to evaluate, that Davis reviews the experimental work which has been done.

The paper first considers the favorable and then the unfavorable evidence; then the intraperitoneal use; the drug as a skin disinfectant; effect in edema; use in wounds; its precipitation by local anesthetics; and the reaction of the body to mercurochrome intravenously.

Seventy-six references are thus abstracted.

Davis concludes that there is experimental evidence of the value of mercurochrome—220 soluble intravenously in the treatment of septicemia and other infections. Other equally convincing experimental results point to the fact that it is not bactericidal in blood, and that its use is not unattended by danger. Many clinical reports show miraculous cures, others have no benefit, and in some it has probably hastened death. Therefore, treatment with mercurochrome must still be considered in the experimental stage. Because of its dangers it should not be used indiscriminately and should be reserved for desperate cases.

Mercurochrome is dangerous intraperitoneally because of the local irritant action and because of the often very severe general reaction.

If used in wounds, sinuses, or serous cavities its dose should be limited to 5 mg. per kilogram of body weight, as it is easily absorbed, and if too much is used, it may lead to severe reaction or stomatitis.

The alcohol-acetone-aqueous solution of mercurochrome recommended by Scott and Hill is a very satisfactory preoperative skin antiseptic. It should not be injected into the nose, urinary bladder, vagina, and so forth, however, along with a local anesthetic, as this will give a precipitate.

ANEMIA: The Relation of Anemia, Primary and Secondary to Vitamine A Deficiency, Koessler, K. G., Maurer, S., and Laughlin, R. Jour. Am. Med. Assn., August 14, 1926, lxxxvii, 476.

The opinion that the toxemia responsible for the symptoms of pernicious anemia is of intestinal origin is chiefly based upon the prominence of the gastrointestinal symptoms of the disease.

The authors believe that toxic substances or toxins are present in the intestinal tract in many instances but are not absorbed or are destroyed before absorption from the intestinal tract.

The problem seems to center around the query, "What are the conditions under which the normal viability and impermeability of the intestinal wall is lost?" Their belief is that a long standing deficiency of vitamine A may be responsible.

They regard pernicious anemia as, at least in certain cases, an intoxication through bacterial poisons formed sometimes by the colon bacillus, at others by the streptococcus and Welch bacillus.

They conclude, from their experiments, that:

1. Blood regeneration cannot take place without the presence of vitamine A.

2. The addition of vitamine A to the diet of animals, long depleted in their vitamine A reserve, brings about rapid formation of new blood cells.

3. The rate and intensity of the blood regeneration is a function of the quantity of vitamine A added.

4. A condition similar to human pernicious anemia has been produced in experimental animals.

5. A definite relationship exists between a state of chronic vitamine deficiency and certain anemias.

6. The routine use of a rationally balanced diet which has proved itself thus far of decided value in the blood regeneration of patients suffering from severe anemias, aplastic as well as erythroblastic, is the most promising procedure in the treatment of certain anemias, especially pernicious anemia.

SPIROCHETA PALLIDA: Are There Immunologic Strains of Spirocheta Pallida? Kolmer, J. A., Weiss, D., and Richter, C. Jour. Infect. Dis., April, 1926, xxxviii, No. 4, p. 378.

An attempt to demonstrate by cross-agglutination and complement-fixation tests the existence of immunologically distinct strains of S. pallida.

No evidence of the existence of such was obtained in the study of six strains.

On the contrary the results may lend some confirmation to the view that in so far as experimental syphilis of the rabbit is concerned, the localization of Spirocheta pallida and the subsequent course of the disease are largely influenced by the virulence of the organism and method of inoculation as well as by the susceptibility of the host and the efficiency of its defensive reaction. Probably the same or similar factors are operative in syphilis of human beings without involving the question of strain specificity or "selective tissue affinity" or the infecting spirochetes.

CARCINOMA: The Mechanism of Cancer Metastasis, Burrows, M. T. Arch. Int. Med., April, 1926, xxxvii, 453.

Metastases in cancer are not the result of a simple migration of cancer cells from the cancer to distant organs. Metastases are primarily the result of the spread of a liquid substance from the main tumor mass. This substance spreads over surfaces. It is liberated through a digestion of cells in the center of the mass of cancerous tissue. This digestion is not an autolysis resulting from the absence of oxygen, but the result of an excess of the growth stimulating substance, a product of the cell's oxidation. The fluid is rich in growth stimulating substance. This fluid stimulates not only the cancer cells to grow but also the normal cells. The cancer cells already adapted to it respond more quickly. In their growth they then remove the nutrition and necessary substances from the other cells and destroy them.

This type of reaction may not always occur. As is well known the normal tissue may undergo malignant transformation. Such has been seen frequently in transplanted cancers of animals. These transformations are the result of a sufficiently long action of this fluid.

WASSERMANN: Wassermann Reaction in Rabbit Syphilis, Wakerlin, G. E., and Carroll, P. H. Jour. Infect. Dis., April, 1926, xxxviii, No. 4, p. 327.

The Wassermann reaction in rabbit syphilis is an index of tissue-spirochete interaction and is not a criterion of the presence or absence of the spirochete.

The Wassermann reaction is consistently positive in about 99 per cent of the cases of active rabbit syphilis following intratesticular inoculation.

The appearance of the positive Wassermann reaction in rabbits affected with syphilis may be completely suppressed by the institution of adequate treatment in the clinically active but prepositive Wassermann stage.

ANAEROBIC INFECTION: Anaerobic Infection: The Process, Dayton, N. A. Boston Med. and Surg. Jour., June 3, 1926, cxciv, No. 22, p. 1032.

The process of infection by gas bacilli depends on conditions which depress the vitality of the tissues and produce a rupture in the normal defenses. This interference may be accomplished by, (a) the introduction of toxin or substances such as calcium salts, the colloids or sterile distilled water, (b) any factor withdrawing the defenses of the blood from the site of injury, such as continued cold, shock, and mechanical or surgical interference with the circulation of the part.

The toxins are two in number and have a local and a hemolytic action, one extending the tissue injury and the other attacking the red cells by lysis. The toxins have a selective action on the suprarenal glands resulting in a complete paralysis of these organs.

The process in infections arising from the gastrointestinal tract differs decidedly from that of a tissue injury. The bacilli have difficulty in establishing themselves and seek an already existing pathologic lesion as a locus of attack or as a means of entering the blood stream. Anaerobic infections have been associated with gastric and typhoid ulcers, gall bladder disease, abscess and carcinoma of the liver and inflammations of the appendix.

The long continued presence of the gas bacilli in the blood stream without the terminal symptoms suggests a chronic infection. This is significant as the blood picture of chronic gas bacillus infection bears a close resemblance to that of pernicious anemia.

URINE SEDIMENT: The Number of Formed Elements in the Urinary Sediment of Normal Individuals, Addis, T. Jour. Clin. Invest., June, 1926, ii, No. 5, p. 409.

The rate of excretion of casts, red blood cells, and white blood and epithelial cells was determined in seventy-four medical students under conditions favorable to the preservation of these urinary constituents. The following results were obtained:

RATE OF EXCRETION PER TWELVE HOUR PERIOD

	AVERAGE	LOWEST	HIGHEST
Casts	1,040	0	4,270
Red blood cells	65,750	0	425,000
White blood and epithelial cells	322,500	32,400	1,835,000

SYPHILIS: Malaria in the Treatment of General Paralysis, Report of Cases, Ridgeway, R. F. L., and Green, E. M. Atlantic Med. Jour., May, 1926, xxix, 544.

Thirty-four patients suffering from general paralysis who were treated by inoculation with blood from one having malaria of the tertian type.

Twenty-six of these patients recovered from the inoculated disease, 7 died, and 1 failed to become infected. Of the 26 patients, 5 were not benefited by the treatment, 9 were improved in greater or lesser degree, and 12 exhibited complete remissions. Seventeen of the number have been paroled from the hospital and 16 are still at home, many of them having reengaged in their former occupations.

The results obtained in the series of cases prove that there may be no correspondence between the mental improvement and the changes in the physical signs of the disease nor in the serologic picture. Several of those manifesting complete remissions exhibited no favorable modifications in these respects. In only a small proportion of the cases did the Wassermann tests of the blood and the spinal fluid show changes which could be attributed to the malarial process. While physical signs were favorably modified in a number of instances, in no case did they all show a return to the normal.

BLOOD: Normal and Pathologic Fragmentation of Red Blood Cells; The Phagocytosis of These Fragments by Desquamated Endothelial Cells of the Blood Stream; The Correlation of the Peroxidase Reaction with Phagocytosis in Mononuclear Cells, Doan, C. A., and Sabin, F. R. Jour. Exp. Med., June, 1926, xliii, No. 6, p. 839.

There is constantly some breaking down of the red cells in the circulation by fragmentation.

The fragments of red cells, as well as whole red cells, are phagocytized and destroyed by clasmatocytes or endothelial phagocytes.

When there is an increase in fragmentation in abnormal or pathologic states, desquamated endothelial cells of the blood stream, as well as the clasmatocytes of the tissues, increase proportionately and take in these fragments. These cells are to be distinguished from eosinophilic leucocytes by the nature of their granules, by the type of motility of the cells, and by a negative peroxidase test.

The desquamated endothelial cells, clasmatocytes, in the circulating blood are positive to the peroxidase test only when they have taken in positive material.

The monocytes show marked variations of the oxidase reaction in different species and to different technics. With the Sato and Sekiya technic most monocytes of human blood are positive, while most of them in rabbit blood are negative, but both positive and negative reactions are found in both human and rabbit blood.

URINARY SEDIMENTS: The Effect of Some Physiologic Variables on the Number of Casts, Red Blood Cells and White Blood Cells and Epithelial Cells in the Urine of Normal Individuals, Addis, T. Jour. Clin. Invest., June, 1926, ii, No. 5, p. 417.

Quantitative determinations of the number of formed elements in the urine of normal individuals failed to show that either bodily movements of various types or the ingestion of a large amount of protein in the form of meat had any statistically significant effect.

ANTHRAX: The Immunization of Sheep by Means of Anthrax Bacilli Attenuated with Sodium Chloride, Schilling, S. J. Jour. Infect. Dis., June, 1926, xxxviii, No. 6, p. 499.

A single injection of a sodium chloride attenuated culture of B. anthracis protected sheep against subsequent infection with a virulent culture; the control animals died in fifty-four hours of typical anthrax. The immunity conferred by the vaccine was general. Vaccination and subsequent inoculation with virulent cultures were performed subcutaneously but in widely separated sites of administration.

Virulent anthrax bacilli may be found at foci of previous infection for some time following the apparent recovery of the infected animal.

The reduced pathogenicity as well as the antigenic properties of the sodium chloride attentuated culture of B. anthracis appeared to remain fairly constant during cultivation on artificial medium.

ANTHRAX: The Attenuation of B. Anthracis by Means of Sodium Chloride and Other Chemicals, Schilling, S. J. Jour. Infect. Dis., April, 1926, xxxviii, No. 4, p. 341.

It was found that sulphuric acid and copper sulphate hydrolyze agar when added to this medium, even in such dilute concentrations as would not be expected to inhibit growth of B. anthracis.

The addition of 4.5 per cent sodium chloride and the addition of 1 per cent potassium ferrocyanide to standard agar appears to represent about the maximum concentration of these chemicals which may be used without completely inhibiting the growth of the anthrax bacillus. The growth-inhibiting concentration of sodium hydroxide is about 0.15 per cent.

An increased tolerance to sodium chloride, potassium ferrocyanide and sodium hydroxide could be noticed in successive transfers of the anthrax bacillus, as judged by the production of a more luxuriant growth.

After growing the anthrax bacillus for seven weeks on agar containing 1 per cent potassium ferrocyanide, and for the same length of time on agar containing 0.15 per cent sodium hydroxide and testing the culture by inoculating guinea pigs, no decrease in virulence of the organism could be detected.

After growing the anthrax bacillus for six weeks on agar containing 5 per cent sodium chloride, marked attenuation of the anthrax bacillus was demonstrated by guinea pig and rabbit inoculation.

Attempts to immunize guinea pigs with the NaCl attenuated culture failed. Presumably this was because sufficient intervals of time were not permitted to elapse between injections.

It was found that rabbits could be successfully and safely immunized by the use of the culture attenuated by growing on 5 per cent sodium chloride agar, so that they withstood the injection of virulent cultures of the anthrax bacillus in quantities which are regularly fatal to normal animals.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Obstetrics**

ONE volume of the Oxford Medical Handbook Series. The purpose of this series is to deal shortly with the fundamental principles which underlie various subjects, and to illustrate these by their proper application in general practice. This volume gives a brief and concise presentation of the subject, including the physiology and pathology of reproduction, the management of pregnancy, of normal labor and of abnormal labor.

Laboratory Outlines in Bacteriology and Immunology†

A LABORATORY guide for the use of instructors in outlining their courses in elementary bacteriology and one that may well be used by the student as a manual which must be supplemented by personal instruction. The work as outlined provides for instruction over a period of one year but is so arranged that the material may be utilized for courses of much shorter duration. In addition to the usual elementary bacteriologic laboratory technic and study of representative microorganisms, a section is devoted to serologic technic including vaccine preparation, hemolysis, bacteriolysis, complement fixation, flocculation tests, the colloidal gold test, phagocytosis, toxins and antitoxins, and hypersensitivity.

It should find a wide usefulness in its field.

Birth Control‡

A SYMPOSIUM by various authors on facts relative to birth control, which should be in the hands of every physician. This is not a disquisition on the relative value of contraceptives but is a clear exposition of the problem as it stands today and a consistent argument for the adoption of some satisfactory method of birth control. The authors take no definite stand as to what methods shall be preferable.

The human race has been here at least 400,000 years. In those thousands of generations the population of the globe had increased up to 850,000,000 in 1830. Within the intervening century this population has been doubled. It is estimated that one hundred years from now there will be three and one-half times the present population, or about five billions. It has

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*Obstetrics. By John S. Fairbairn, M.A., B.M., B.Ch. (Oxon.) F.R.C.P. (Lond.); F.R.C.S. (Eng.) Cloth. Illustrated. Pp. 221. Price $1.75. Humphrey Milford, Oxford University Press.

†Laboratory Outlines in Bacteriology and Immunology. By John F. Norton, Ph.D., and I. S. Falk, Ph.D. Cloth. Pp. 114. The University of Chicago Press, Chicago, Ill. 1926.

‡Birth Control—Facts and Responsibilities—A Symposium Dealing with this Important Subject from a Number of Angles. Edited by Adolf Meyer, M.D. Cloth. Pp. 157. Price $3.00. The Williams and Wilkins Company. 1925.

been estimated that five billion people are the most that can be fed if every tillable acre in the world is tilled as well as we know how to do it.

Those, whose religion opposes birth control, insist that we are commanded in the Bible to increase and multiply and replenish the earth. They seem to forget that this command has already been obeyed in full, and that the command was given as an emergency procedure after a terrible flood in which all but eight people had been drowned. Since that time the population of the globe has been multiplied two hundred and fifty million times.

We hear much of the declining birth rate. This sounds bad until we realize that concomitant with this the death rate is shrinking still faster due to improved methods of preventive and curative medicines.

The antagonist often replies that by the time we become over-populated new methods of synthetic food preparation will relieve the situation. They do not realize that not a single plant of dietary importance has been brought into cultivation within historical times. Prehistoric man discovered them all and now that there are no unexplored regions the probability of new boons like corn and potatoes is small. The limits of production will be expanded, the ravages of insects and fungi reduced but neither genetics nor chemistry can be expected to furnish a final solution to the old malthusian proposition.

War is but a temporary check to population. This requires no argument in substantiation, only census figures. The same is true of pestilence and famine.

Sentimentalists insist that all are born equal. The farmer knows that his animals are not born equal and he saves the well-bred for breeding purposes and destroys the others. Human mating today is controlled chiefly by propinquity, religion, race, social position and personal attraction. Eugenics is not a dead science nor is it purely theoretical. Some day it will again come to the fore in a more practical aspect.

*Obesity**

DR. LEONARD WILLIAMS has written a very readable tirade against fat people. It is for lay consumption primarily. The unlovely condition called corpulence has been divided into three stages known respectively as the enviable, the comical, and the pitiable. He denies a place to the enviable obese. No degree of obesity is enviable.

The author draws widely on known facts of nutrition and metabolism and endocrinology and intersperses with them some of his own theories.

He has far less patience with the obese man than with the obese woman. Obesity in the male is, in his opinion, nearly always due to gourmandism and is therefore disgusting. Obesity when it occurs in the female is, in his opinion, usually endocrine in origin and therefore excusable. The fairer sex may, therefore, read the volume without gross injury to personal pride. Indeed, the author rather retraces his steps after his flagellation of the male for he lauds the slight corpulence of motherhood while decrying the boyish figure of the cocotte. In the former small accumulations of fat are physiologic in preparation for the increased metabolic drain of pregnancy and the maternally inclined woman, who looks upon sexual congress as a means to an end, is allowed to put on a little excess adipose tissue without criticism while the latter in whom the sexual act is an end in itself is severely criticised for keeping her figure slim so that it may be attractive to the male. This volume should be a source of gratification to the German hausfrau.

The physician may read this volume for relaxation and in it will find many good convincing arguments for use in his consultation work.

*Obesity. By Leonard Williams, M.D. Author of "Minor Maladies" and "Middle Age and Old Age." Cloth. Humphrey Milford. Oxford University Press

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EDITORIALS

The Etiology of Granuloma Inguinale

GRANULOMA inguinale is described by Fox[1] as a chronic, infectious, ulcerative process usually, but not necessarily, involving the genitalia or neighboring parts, showing little or no tendency to spontaneous healing, and yielding to treatment with tartar emetic (antimony and potassium tartrate).

Originally recognized and first described as "serpigenous ulceration of the genitals" by McLeod in India in 1882, it was first isolated as a clinical entity by Conyers and Daniels[2] in 1896, and since that time has been described under various names in various localities.

While long regarded as a tropical disease, the reports of numerous investigators have shown a rather wide geographical distribution and it is of particular interest to note that recent reports indicate that this disease is endemic in various parts of the North and South and is much more prevalent in the United States than is generally supposed.

There has been, and still is, much discussion as to the etiology of this infection. By many observers for a long time the inclusion bodies first described by Donovan[2] in 1905 and now generally known as "Donovan bodies" have been regarded as the etiologic agent.

The occurrence of these bodies is an accepted fact in the lesions of this disease and the present controversy concerns their nature rather than their relation to the lesions.

There is some reason to question the identity of these bodies with the genus Leishmania and various workers have presented more or less convincing evidence that the bodies seen in this disease are not protozoal forms but bacteria of the B. mucosus capsulatus group.

On the one hand there is, as yet, no convincing evidence of the cultivation of Leishmania, and no evidence in any other bacterial disease of the specific action of a synthetic drug; and, on the other, some very suggestive reports are available of inoculation experiments with cultures isolated from granuloma lesions.

Lynch,[4] for example, has cultured an organism morphologically similar in smear and culture which he regards as of etiologic importance and which he further believes distinct from the Donovan bodies of whose etiologic relationship to granuloma inguinale he is not altogether convinced; Campbell[5] suggests that the Donovan bodies may be secondary invaders, while not committing himself as to the etiologic importance of the encapsulated organism isolated from his cases; and numerous similar reports can be found in the literature.

It may be granted that encapsulated and more or less pleomorphic bacteria can be cultured from a large percentage of cases; that they not infrequently appear morphologically indistinguishable from the bodies seen in direct smears from the lesions; and that, apparently, these bacteria are members of the group of which B. mucosus capsulatus is the type.

Their relationship to the production of the disease, however, awaits extensive experimental inoculation studies of which, as yet, there are not very many reports.

Cornwall and Peek,[6] however, have recently described an organism which they believe does not belong to the B. mucosus capsulatus group and which, in old cultures, reproduces exactly the morphology of the Donovan bodies.

In a second paper[7] they report inoculation experiments on rabbits recovering the same organism from the lesions produced; and Goldzieher and Peek[8] continuing studies on the same organism report studies supporting their belief in the etiologic relationship of this organism, for which they propose the name Bacillus venereogranulomatis, by complement-fixation and allergic tests as well as animal inoculation.

The question remains an unsettled one worthy of extensive study.

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—*R. A. K.*

Some Recent Contributions on the Treatment of Pneumonia

THE last two or three years may be characterized as a period in which we have been becoming more intimately acquainted with the pneumococcus as a germ. Careful bacteriologic, immunologic, and chemical studies of the pneumococcus cell are being made and while many of the observations recorded have no direct or apparent bearing on the treatment of pneumonia, the result will be an accumulation of knowledge, some of which will be applied in practical therapeutics. This intensive study is being carried on particularly by Avery, Dochez, and their coworkers at the Rockefeller Institute, by Felton and Bailey at Harvard and by Falk and Jacobson at the University of Chicago.

Work at the Rockefeller Institute indicates that the fixed type pneumococci may be divided into two categories, *type specific* or S strains and degraded or R strains. Both groups are present in all three of the fixed types. The type specific pneumococci retain their capsules. The degraded ones have lost them. The type specific antigens appear to be associated with these capsules and are carbohydrate, polysaccharide, in nature. Reimann finds that immune sera for the degraded R strains cross agglutinate. A serum immune to a Type II R strain will also agglutinate other fixed type R strains. It will not agglutinate fixed type encapsulated S strains, not even of Type II. Apparently in immunization against pneumococci two varieties of agglutinins are produced, a general one for the pneumococcus protein and a specific variety against the type specific antigen. While the R immune sera do not agglutinate the type specific pneumococci, they do precipitate the free protein of these S strains *after* autolysis. Here the pneumococcus protein has apparently been liberated from the type specific antigen.

The R strains are serologically identical. They evoke *species—specific* antibodies and not *type-specific* antibodies.

The recognition of type specific and degraded pneumococci explains confusing cross reactions which have been observed and might conceivably have some bearing on the varied results that have been reported in the literature following treatment with Type I immune serum. A Type I serum produced with degraded Type I pneumococci will have little or no effect against a type-specific Type I pneumococcus.

Sia finds that the specific soluble substance from a fixed pneumococcus increases the virulence of an otherwise avirulent pneumococcus. This specific soluble substance is polysaccharide in nature and is probably related to

or the same as the type specific antigen previously discussed. Pneumococci, either naturally endowed with this substance or reinforced by its addition, become more virulent.

Within the last few years there has been no outstanding alteration in the serum treatment of lobar pneumonia. The results obtained with Type I serum at the Rockefeller Institute still stand, although certain workers have not obtained as convincing results. Perhaps the most outstanding requisite for success is serum administration at as early a date in the disease as possible. More recently Gay and Chickering have produced a concentrated Type II serum which carries with it some promise of results.

Polyvalent, immune sera are of value only in Type I pneumococcus infection, for they do not contain a sufficiently potent concentration of Types II and III antibodies to be of value.

Huntoon has separated the antibodies from the serum proteins. The separation of the immune body from the other serum constituents is naturally an ideal to be attained. He prepares a polyvalent serum and by exposing it to contact with pneumococci effects combination between the antibodies and the antigen. The antigen has become sensitized. The antibody has become absorbed. The bacteria are then centrifuged and washed with salt solution until the serum is entirely removed. The antigen-antibody combination is next emulsified and an appropriate amount of alkali added. The mixture stands overnight, during which time the antibody is set free in great part from combination with the antigen. The latter is then thrown out by centrifugation.

The supernatent fluid contains the antibody with agglutinins and probably a slight portion of antigen still attached. This is further purified and finally filtered through porcelain. There is practically no protein in the solution.

Antibody solution injected intravenously has produced occasional severe reactions which have in one or two instances terminated fatally. For this reason subcutaneous administration was substituted. Oliver and Stoller report their results with the subcutaneous administration of antibody solution. They found that in a study of twenty-three cases two had mild febrile reactions, all had local pain and one developed what appeared to be an extensive cellulitis. Only four experienced subjective improvement and but three displayed objective improvement. The remainder were not benefited. They concluded that pneumococcus antibody solution as prepared at that time (1925) is of less value in the treatment of Type I pneumonia than is Type I serum. It does not prevent extension to other lobes. Their best results were obtained in Type IV infection where the mortality was 10 per cent.

They found that subcutaneous administration did not sterilize the blood stream. This apparently is due to the fact that antibodies either do not appear, or appear but slowly within the blood stream.

Huntoon therefore has experimented further with intravenous administration. He finds that the severe reactions are apparently usually associated with extraneous bacterial contamination. This has been greatly obviated by manipulation at low temperatures. He has also perfected a method of anti-

body concentration. It can now be concentrated about 40 times with the result that the dose is 5 or 10 c.c. intravenously instead of the former 50 to 100 c.c.

Baldwin and Cecil report promising results in the treatment of Types I and II pneumonias, with Felton's concentrated serum.

The vaccine treatment of pneumonia is, as usual, before us for consideration. Rosenow and Hektoen in 1913 recommended the administration of partially autolyzed pneumococcus antigen. This appears not to have been followed up in recent years. Alexander Lambert reports treatment with a mixed bacterial vaccine containing in each c.c. 200 million Pfeiffer bacilli, 100 million pneumococci, 100 million streptococci, 200 million Micrococcus catarrhalis, and 200 million each of Staphylococcus albus and aureus. There are in all 160 strains of bacteria. He injected from 1 to 2 c.c. of this vaccine intramuscularly every six hours until the temperature had reached 99 and then every twelve hours for two or three days, and finally once daily until symptoms had entirely subsided. He reported no reaction from these treatments. Two hundred twenty-one cases so treated were compared with 286 control cases observed during the same season. The mortality among the treated was 19 per cent as contrasted with 37 per cent among the controls. When treatment was started within the first forty-eight hours, the death rate was reported as 5.8 per cent; within the first seventy-two hours as 9.8 per cent. He records a diminished severity rather than a shortening of the course of the disease, following this mode of treatment.

It is a matter of ancient observation that patients with lobar pneumonia are more comfortable when in the open air. Oxygen treatment received its first impetus following its application in 1917 by Haldane in the treatment of acute pulmonary edema. Various apparatus have been devised for the oxygen administration, some simple, some intricate, some apparently efficient, others inefficient. Barach delineates the present status of oxygen therapy. For satisfactory results the inspired air should contain from 30 to 60 per cent oxygen. It is not safe to breathe for a long period air containing over 70 per cent oxygen. Best results are to be anticipated when the oxygen concentration is between 40 and 50 per cent. Below 30 per cent oxygen is ineffective. The ordinary funnel method delivers oxygen in a concentration of about 24 per cent, scarcely 5 per cent increase over atmospheric concentration. The proper use of the nasal catheter will deliver oxygen into the nasopharynx at a concentration of 30 per cent. This is when the oxygen is being run at the rate of about 2 liters per minute. With the "bubble method" usually but 1 liter is delivered per minute.

There are many potential sources of error in nasal catheter administration. The catheter may be in the anterior portion of the nose, not in the nasopharynx. It easily becomes clogged and should therefore have several perforations in the tip and should be cleaned every four hours. The patient may breathe entirely through the mouth. Too abundant oxygen flow may be irritating to the patient.

The Barach rebreathing apparatus, equipped with soda lime for the absorption of the expired carbon dioxide, will deliver 40 per cent oxygen con-

centration when the gas is being run at a rate of 1 liter per minute. This results in a distinct saving of oxygen.

The tent methods are apparently most efficient, delivering from 40 to 60 per cent concentration. Guedal has devised a simple, cheap oxygen tent made with barrel hoops cut in two and arranged criss-cross to serve as supports and covered with a single layer of muslin. This is placed over the patient's head and oxygen is delivered through a tube the end of which is in the neighborhood of the patient's face. No provision is made for removal of carbon dioxide, although a small aperture is sometimes left along the base for ventilation. Delivering 3 liters of oxygen per minute, the author maintains a concentration under the tent of about 35 per cent. His criterion for administration is the extent of cyanosis of the finger nails. Oxygen is given until the nails are no longer cyanosed. The oxygen flow is then adjusted at such a rate as will keep the nails free from blueness.

In this work commercial oxygen in high pressure cylinders is more economic than low pressure medicinal oxygen. It may be purchased in 110 or 220 cubic foot cylinders to which are attached a pressure gauge so graduated as to indicate the flow of oxygen in liters per minute. The expense with the Guedal apparatus, which is rather extravagant of oxygen, using on an average of 3 liters per minute, runs from $6 to $8 per day.

No extravagant claims are made for oxygen in reducing mortality from lobar pneumonia. Barach characterizes the treatment as supportive but not curative. It reduces the cyanosis, dyspnea and restlessness. The respiration and pulse are often favorably influenced and delirium if present is usually decidedly lessened.

Diathermy is a relatively recent departure in pneumonia therapy. The pneumococcus will not long stand a temperature of 106° or over. As is well known the application of diathermy increases the temperature of the tissue between the electrodes. Stewart claims for diathermy an improvement in the circulation through the hepatized lobe, improvement in coronary circulation and increased phagocytic activity. He finds that after diathermy the pneumonia patient experiences a change in the character of the respiration rather than in the rate, the respiration being deeper and freer, due perhaps to the diminished pleural pain or perhaps in part to the other changes attributed to the treatment. He states that the relief from cyanosis is constant, ascribing it to better functioning of the right ventricle and perhaps to better aeration in the lungs. The temperature falls by lysis rather than by crisis. Diathermy so far has not prevented extension into other lobes. Stewart describes a mortality of 15 per cent as contrasted with 43 per cent in control cases. There were 254 in the former group and but 31 in the latter. The majority were not typed. Walsh observed a 12.3 per cent mortality in 95 diathermy treated cases and 20.3 per cent among 59 who did not receive this treatment.

The drug treatment of lobar pneumonia is practically in statu quo as compared with the last few years. Optochin, ethylhydrocuprein, described by Moore in 1915, has developed little farther. No literature of great importance has appeared on this remedy within the last two years. This quinine

derivative appears to be directly pneumococcidal but is too toxic, producing amblyopia in several cases, permanent contraction of the visual fields in a few and permanent blindness in one. Otherwise this remedy held considerable promise, the pneumonia mortality being around 10 per cent.

Digitalis continues to hold its place. As in the past there is no unanimity as to whether it should be given throughout the course of the illness or withheld until specific indications arise. With the methods for rapid digitalization now available its administration to an otherwise normal heart is as a rule not necessary. Where evidence of myocarditis exists, digitalis should be instituted without delay. Lawrence studying 1000 cases at Camp Devens found x-ray evidence of enlargement of the heart early in pneumonia. Robinson at the Atlanta meeting of the Southern Medical Association reported experimental evidence showing that digitalis diminishes the size of the heart, thereby increasing its output and its efficiency. In summarizing the digitalis problem we may say that the proper time for its administration remains optional.

Mercurochrome intravenously or intraperitoneally has been recommended for pneumonia in children. Hoppe and Freeman treated 23 cases of lobar and bronchopneumonia with mercurochrome in the usual dosage. The mortality among these children was 8.5 per cent as against 39 per cent in the controls. The duration of illness in the former was 6.5 days as contrasted with 16.5 days in the latter.

Spectacular, if not extravagant, claims are made by Nott for the potassium permanganate treatment of lobar pneumonia. Two grains of the pure chemical are dissolved in one and one-half pints of warm water and this is given slowly per rectum in three or four ounce quantities every two and one-half to four hours for the first twenty-four to thirty-six hours. After this it is administered twice daily for three days, then once a day for three days. The course of the illness naturally determines the frequency and length of administration. Forty cases have been so treated with a 5 per cent mortality. Nott claims for this method a remarkable sedative action, the clearing up of blood from the sputum within a few hours after its first administration, and a rapid temperature fall by lysis. He makes no attempt to explain the phenomenon. If the results are as good as the author believes, they are deserving of substantiation with more carefully controlled clinical observation. He also administers thyroid extract by mouth but doubts whether this is a factor in the good results.

In reviewing the advantages claimed for these various therapeutic procedures, in terms of the mortality rate, the great variation in both treated and control cases is evident. In view of the differing severity of the disease at different seasons and in different local prevalences, it is necessary each time to run a control series treated under analogous circumstances. Even then, however, pneumonia is a disease with so many varying factors and complications that few series treated by any one method are sufficiently large to eliminate the influence of these variables.

Thus the type of pneumococcus infection, a factor of great importance, is sometimes not mentioned. Some of the reports which we have reviewed

make no mention of the causative organism. Type I pneumococcus is associated with a rough mortality of 25 per cent, Type II of 32 per cent, Type III of 45 per cent and Type IV of 16 per cent. A different relative proportion of type infections between the test series and the control series will vitiate the results. Probably the best figure that we have on general pneumonia mortality is that of Wells who reports a 20.4 per cent death rate in 465,400 cases of pneumonia. Here the number is sufficiently large so that the complicating factors mentioned have become well nigh negligible.

The great discrepancy in the death rates reported above makes comparison difficult or impossible. This brings us to the conclusion that there is at present no "all around" specific for lobar pneumonia. Each case must be individualized. The basic principles are still important: rest, both mental and physical, abundant fluids, a light diet, particular care to avoid abdominal distention, digitalis where indicated, caffein as a respiratory stimulant occasionally, abundant fresh air, and a careful watch for complications. Of these probably the most important is rest, natural if possible, induced if necessary.

Beyond this the doctor treating the individual case will be interested not so much in a series mortality as in selecting those of the more recent therapeutic measures which are calculated to give the patient greater subjective and objective relief from the individual symptoms as they arise. Some of the measures described above will fall within this category.

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